Chapter 6

Agriculture

Contents

	Page
Introduction	161
Animal Agriculture	162
Diagnosis, Prevention, and Control of Animal Diseases	162
Animal Nutrition and Growth Promotion	167
Genetic Improvement of Animal Breeds	168
Commercial Aspects of Biotechnology in Animal Agriculture	169
Conclusion	171
Plant Agriculture	172
Improvement of Specific Plant Characteristics	174
Uses of Microorganisms for Crop Improvement	181
Conclusion	184
Commercial Aspects of Biotechnology in Plant Agriculture	185
Priorities for Future Research	186
Animal Agriculture	186
Plant Agriculture	186
Chapter preferences	188

Tables

Table No.	Page
27. Viral Animal Diseases and Potential Vaccine Production	164
28. World and U.S. Sales of Growth Promotants	167
29.Global Animal Health Product Markets	170
30.U.S.Producers of Animal Health Productsts	170
31. Sales of Major U.S. Animal Vaccine Products, 1981	171
32. Major Producers of Animal Vaccines Sold in the United States	171
33. Plant Resistances of Economic Value	177
34.U.S. Soils With Environmental Limitations	177
35. Distribution of Insurance Indemnities From Crop Losses in the United States From 1939 to 1978	177
36. Examples of Secondary Plant Products of Economic Value	179
37.Importance of Basic Research (Model Systems) on Nitrogen Fixation	183

Figure

Figure No.	Page
16.Steps To Create a New Variety of Plant by Using Biotechnology	174

Chapter 6

Agriculture

Introduction

As the world population grows, agriculture will need to provide more and more food. Biotechnology may yield methods and products that improve agriculture in many ways. In animal agriculture, biotechnology offers promise in the following areas:

- *diagnosis, prevention, and control of animal diseases* with the use of monoclonal antibody (MAb) technology to diagnose, monitor, and better understand disease and the use of recombinant DNA (rDNA) to expand the pharmacopoeia of vaccines and other animal health products;
- *animal nutrition and growth promotion* through the use of growth hormones and feed additives to improve animal feed usage and animal health; and
- *genetic improvement of animal breeds* by using MAb and rDNA technology to better understand the bases of animal productivity and disease resistance and by the direct transfer of "beneficial" genes from one animal breed to another.

Though the potential for using biotechnology to improve animal agriculture is exciting, the commercial feasibility and actual impacts of using biotechnology in this area at present remain largely speculative. In some cases, existing animal health products may be replaced by improved, biotechnologically made materials. In other cases, entirely new products may become available to solve formerly intractable problems. In almost all instances, efficacy, safety, and practicality must be demonstrated for each new product. Only a few products for practical use in animal agriculture have been produced to date, so the success of biotechnologically produced compounds compared with conventionally made products remains to be demonstrated. For many animal agriculture products made with new biotechnology, the speed and scale of adoption by producers will be determined by the ease with which the products can be integrated into existing production systems (20).

In addition to the potential applications of biotechnology in animal agriculture, there are several potential applications in the area of crop improvement. The potential applications generally fall into two categories:

- *improvement of specific plant characteristics*, for example, through the introduction or manipulation of genes that confer resistance to disease and environmental factors, that increase the amount and quality of primary and secondary products from plants, that enhance plant growth rate, or that increase photosynthetic efficiency; and
- *genetic manipulation of micro-organisms*, for example, to enhance the process of nitrogen fixation, to produce insecticides, or to suppress disease or promote growth in plants.

The genetic manipulation and modification of plants presents some special challenges, but research is proceeding rapidly. There is a great deal of research interest at present in the use of biotechnology to improve plant resistances to disease and environmental factors. If plants are made more resistant to disease and certain environmental factors, greater crop yields or a reduction in the cost of crop production may result. Furthermore, unlike most plant traits, some of these specific crop improvements may be accomplished with only one or a few gene modifications. It is likely that there will be considerable research progress in this area in the next 5 to 10 years.

The applications and commercial aspects of biotechnology in animal and plant agriculture, respectively, are discussed in more detail in the next two sections of this chapter. A separate section at the end of the chapter indicates priorities for future research in each of these areas.

Animal agriculture

The commercial use of biotechnology in animal agriculture is affected by several often-contradictory forces. Favorable forces include the extensive use of animals as test models in basic research and, as is discussed in ***Chapter 15: Health, Safety, and Environmental Regulation,*** less stringent regulatory approval processes for animal health products than for pharmaceutical products intended for human use. Because animals are used during the development of pharmaceutical and biologic products for humans, veterinary medicine stands to benefit from biotechnology research and development (R&D) such as that described in ***Chapter*** 5: ***Pharmaceuticals.*** Biotechnologically made products for use in animal agriculture, such as MAb diagnostic products, growth hormones (GHs), and vaccines, are becoming available on a limited basis.

Among the factors that inhibit commercial applications of biotechnology in animal agriculture is the fact that the low value-added nature of individual farm animals limits veterinary costs per animal, veterinary medicine sales, and funding for veterinary R&D. In addition, some biotechnologically made products do not suit current animal husbandry practices. Commercialization of at least one rDNA-made vaccine, the vaccine for foot-and-mouth disease (FMD), for example, awaits successful applied research results to achieve protection against several strains of the disease, fewer dosage requirements, lower costs, and other improvements that make the vaccine amenable to animal husbandry practices in the developing nations where FMD exists (20).

Biotechnological developments in the areas of animal disease control, animal nutrition and health, and genetic improvement of animal breeds are discussed further below. Distinctions between the use of biotechnology to expand fundamental knowledge and to develop specific products for commercial use are noted.

Diagnosis, prevention, and control *of animal diseases*

Losses due to animal diseases exceed hundreds of millions of dollars yearly in the United States, * giving strong impetus to efforts to improve animal health. A combination of the techniques of biotechnology is being used to better understand viral, bacterial, protozoan, and parasitic infections that affect animal productivity throughout the world, MAbs, for example, are being used as research tools to gain a better understanding of the molecular biology of animal diseases. MAbs may also be used for diagnosis of diseases, for monitoring the efficacy of drugs, and for providing short-term passive immunity against animal diseases. In addition, recombinant DNA technology and polypeptide synthesis maybe used to develop vaccines for long-term immunization against certain animal diseases.

MONOCLINAL ANTIBODY DIAGNOSTIC PRODUCTS

The diagnosis of animal diseases can be accomplished by the identification in the laboratory of specific antigens displayed by the infectious agent. As discussed in *Chapter 3: The Technologies,* MAbs that recognize specific antigens can be prepared readily. MAbs for several animal diseases are now being made, and in vitro MAb diagnostic products for a number of animal diseases may be used in the near future. MAb-based diagnostic tests are currently being developed for bluetongue, equine infectious anemia, and bovine leu - kosis virus. Furthermore, diagnostic MAbs are be-

● **"Animal losses" are described by a number of parameters, including dollar value of animals lost, losses in productivity due to morbidity, and value of potential progeny lost due to sickness** or **death of breeders. In this report, the dollar value of animals actually lost to disease (as a primary cause of death) is used for the sake of comparison in describing animal diseases. These estimates are based on data collected for U.S. Department of Agriculture's Animal and Plant Health Inspection Service, Veterinary Services,** Hyattsville, **Md., and by Deane Agricultural Services, Inc., St. Louis, Mo.**

ing sought for canine parvovirus, canine rotavirus (a potentially fatal viral diarrhea in puppies), feline leukemia virus, and canine heartworm disease. For MAb diagnostic products to be effective diagnostic tools and hence commercially viable, they must recognize the large variety of disease strains likely to be encountered **(20).**

The acceptance of iMAbs for field use by veterinarians and animal owners remains to be demonstrated. Whether MAb products will have a large role in the diagnosis of specific animal diseases is unclear. Since livestock producers and poultry growers attempt to spend as little money as possible per animal raised, the markets for individual MAb diagnostic tests initially may be limited. Applications of MAb diagnostic as well as therapeutic products initially may be restricted to high-profit animals, animal products for export, and companion animals such as dogs, cats, and horses. Although individual diagnostic kits are not costly, the farmer's narrow margin of return on other animals may prevent the routine use of diagnostic products.

In the future, diagnostic MAbs could substantially assist large-scale disease control programs in both developed and less developed countries **(16). Such reagents might** be used to detect disease in order to select an appropriate vaccine and monitor the level of disease during the course of a control program.

Apart from potentially being used as diagnostic reagents by animal producers, MAbs can be used as purification tools to isolate compounds (antigens) that may prove to be effective animal vaccines. They can also be used to provide "passive immunity" to certain animal diseases. The applications of biotechnology to the development of ani - mal vaccines is described further below.

ANIMAL VACCINES

Prevention **of a number of animal diseases is** being sought **with rDNA subunit vaccines in ef -** forts similar **to human vaccine programs de-**scribed in *Chapter* 5: ***Pharmaceuticals.*** Subunit vaccines may solve some of the problems associated with conventional vaccines. One problem, for example, is that "attenuated" and killed whole vaccines contain the genetic material of the pathogen and therefore have the potential to cause the infection they are supposed to prevent. Subunit vaccines do not contain the pathogen's genetic material and therefore cannot cause infection. Subunit vaccines may also be more stable, more easily stored, and of greater purity than conventional vaccines, but these qualities remain to be demonstrated. Despite their potential advantages, subunit vaccines raise new technical problems, as mentioned above, and these must be overcome if the vaccines are to prove useful in the field (20).

Viral Animal Diseases.—The development of improved vaccines may allow the prevention of several problematic animal diseases caused by viruses (34). Most subunit vaccine research for animals to date has been focused on viral diseases, particularly FMD and rabies, but some of the findings can be generalized to other viral diseases, Table 27 shows some viral diseases in animals against which subunit vaccines may prove effective and economic.

The development of subunit vaccines for FMD is currently receiving much attention from researchers (2). Although the disease is nonexistent in the United States, FMD affects livestock productivity and exportability throughout South America, Africa, and the Far East. The world market for FMD vaccine is larger than that of any other vaccine, either animal or human. In 1981, 800 million doses of inactivated FMD virus vaccine worth $250 million were used (36). Vaccines for all types of FMD commonly encountered exist at present, but these vaccines vary in effectiveness against different FMD field strains. Evolution of new field strains is a continuing problem, because a vaccine may lose its effectiveness against such strains. The impetus for developing a subunit vaccine for FMD is the hope that such a vaccine will offer enhanced protection with greater safety than conventional vaccines. The degree of protection offered, however, will only become clear over the next few years as research and field evaluations progress (9).

Three research groups have cloned the gene that codes for the major FMD viral surface protein (5,14,15). The new biotechnology firm (NBF)*

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

Table 27.—Viral Animal Diseases and Potential Vaccine Production

Disease	Potential for new biotechnology	Company	Current vaccine status	Potential for new vaccine
Viral diseases:				
Foot-and-mouth disease	+	Genentech (U. S. MJSDA (U. S.) Pirbright (U.'K.) Biotech Gen (Israel) MGI (U.S.)[a]	Medium	Replacement
Rabies	+	Wistar Transgene (France) Genentech (U. S.) Inst. Pasteur (France)	Variable	Replacement
Parvovirus:				
Swine	+	MGI	Poor	Replacement
Canine	+	TechAmerica (U. S.)	Medium	Replacement
Bovine leukosis virus	+	MGI	N.A.[b]	Replacement
Bovine papilloma virus	+	MGI	N.A.	Export animals
Rift Valley fever	+	MG1/U.S. Department of Defense	Good	Replacement
Marek's disease (fowl)	+	BRL (U.S.)[c]	Medium	Replacement
Infectious bovine rhinotracheitis	+	MGI	Medium	Replacement
Pseudorabies	+	MGI	Medium	Replacement
African swine fever	+	Spanish Government	None	New product
Rota viruses	−	Vido Institute University of Saskatchewan	None	New product
Bluetongue	+	Bio-Tech Gen. (Israel) USDA	Poor to medium	Export animals
Hog cholera	−	N.A.	Good	Replacement
Newcastle disease	+	USDA	Poor in some areas	Replacement
Bacterial diseases:				
Tuberculosis	N.A.	N.A.	None	New product
Neonatal diarrhea	+	Cetus (U. S.)/Norden (U. S.) InterVet (Netherlands/ Akza (U. S.) MGI	Poor	Replacement
Bacterial respiratory disease	N.A.	N.A,	Poor	Replacement
Anaplasmosis	N.A.	N.A.	None	New Product
Parasitic diseases:				
Babesiosis	+	IMC (U.S.)[d]	None	Replacement
Trypanosomiasis	+	American Cyanamid (U. S.) Genex (U. S.) Hoffmann-La Roche (Switz.)	None	New product
Coccidiosis	+	Eli Lilly (U. S.)	Good	Replacement
Helminithic diseases	+	Merck W. S.)	Fair	Replacement

a MGI _Molecular Genetics, Inc

b N.A = Information not avalable

c ~RL- Bethesda Research Laboratories

d IMC = International Minerals & Chemicals Corp

SOURCE Board of Science and Technology for International Development, et al , "Prlorltles In Biotechnology Research for International Development—Proceedings of a Workshop" (Washington, D C National Academy Press), and the Off Ice of Technology Assessment

Genentech Corp. (U.S.), in collaboration with the U.S. Department of Agriculture (USDA), cloned the DNA that encodes the protein of one strain of FXID into bacteria, made the protein product in large enough quantities for field trials, and tested it at USDA's Plum Island Animal Disease Facility **(14). The FMD subunit vaccine protected animals against infection by the particular strain against which the vaccine was made (although the field** trial was not extensive), but it was less effective than the whole inactivated vaccine. The two other research groups working on a subunit FMD virus vaccine are a Swiss-West German team (University of Heidelberg, Federal Research Institute for Animal Virus Diseases at Tubingen, Max Planck Institute for Biochemistry, and Biogen S. A.) and a British team (Animal Virus Research Institute and Wellcome Research Laboratories) (9).

Cloning of the genes that code for the surface proteins of viruses of fowl plague, influenza, vesicular stomatitis, herpes simplex, and rabies also has been achieved, and the cloned genes may lead to the development of effective subunit vaccines for these animal diseases (2). Cloning projects for virus proteins that cause gastroenteritis, infectious bovine rhinotracheitis, Rift Valley fever, and paramyxovirus currently are underway (2), Different challenges are associated with each project. Rabies projects, for example, have encountered problems with the consistent expression of the surface protein from rDNA plasmids (34). Influenza virus projects, among others, face problems in that the natural viruses spontaneously change their surface proteins to evade the immune system, making the choice of optimal genes for cloning difficult.

Another method being used to prepare new subunit vaccines for animals, aside from the use of rDNA technology, is chemical synthesis of peptides. Synthetic peptides corresponding to part of one viral surface protein of FMD protect test animals against live FMD virus (3), and efforts are underway to prepare synthetic rabies vaccines (28). As noted in Chapter 5: *Pharmaceuficals,* most synthetic vaccines are prepared with the use of MAbs as purification tools, Chemically synthesized peptides ma-y prove useful in rapid screening programs to determine which peptides act as the best vaccines; subsequently, the DNA corresponding to these fragments may be cloned for large-scale production in microbial systems.

Whether produced from rDNA or chemical synthesis, subunit vaccines for viral animal diseases must satisfy several requirements to be effective. In most instances, subunit vaccines must contain antigens from a sufficient number of different strains of virus to offer comprehensive protection against field challenge. The new vaccines must induce a protective immune response to the same or greater degree than conventional vaccines if they are to compete for market shares. Proper dosage and timing of vaccination must be determined. Ideally, the vaccines should be administered in a single injection to be amenable to most husbandry practices throughout the world where animals are dispersed over wide tracts of land. Also, long shelf storage life and stability when stored at room temperature are desirable features of the new vaccines for use in all the countries affected by the particular diseases. *

In addition to subunit vaccines that provide active immunity, MAbs may be used to provide passive immunity against a variety of viral animal diseases. Several MAb-based products currently are being developed. For instance, antirabies MAbs that protect mice from active rabies virus have been made (19). The use of these products, however, is likely to be limited to herds (e.g., dairy animals) where the passive vaccines can be readily and repeatedly administered.

Bacterial Animal Diseases.—The potential for biotechnology in fighting bacterial diseases in animals is less clear than its potential in fighting viral diseases, but several promising advances are currently being made. In developing new methods to prevent these diseases, an understanding of the natural and pathogenic roles bacteria play in domestic animals is important. Numerous types of bacteria are normal inhabitants of both human and animal gut. In general, disease may result when animals, especially those predisposed to infection (e.g., young, weak, or stressed animals), either succumb to pathogenic bacteria or suffer from overgrowths of their own native bacteria. Bacterial infections often occur simultaneously with other infections, including viral invasions. Because of the complexity of most of the currently important animal diseases in which bacteria are involved, the effectiveness of bacterial vaccines produced by biotechnology is difficult to predict.

Bacterial vaccines against colibacillosis (scours), a widespread disease that causes diarrhea, dehydration, and death in calves and piglets, are be-

* At present, the rabies subunit vaccine is most promising in meeting the criteria for becoming a competitive vaccine There appear to be only slight variations in surface protein sequences between rabies virus strains, and these surface proteins elicit large immune responses The RNA encoding several viral surface proteins has been cloned and expressed in *E coli* (34) Questions that remain concerning the efficacy of this vaccine include 1) the need for glycosylation of the rDNA-product for proper functioning (see *Chapter 5: Pharmaceuticals),* and 2) proper delivery systems, primarily to wild animal reservoirs such as skunks and foxes, where rabies proliferates, and to dispersed animal herds such as those in South America, where the death of cattle infected by the bites of rabid vampire bats results in an estimated yearly loss of more than $29 million (34)

ing made with biotechnology. Recombinant DNA technology is used to change bacterial plasmids found in pathogenic strains of enteric bacteria from a virulent to a harmless state. This approach is used by both Intervet (Netherlands) and Cetus Corp. (U. S.) to prepare vaccines against colibacillosis. These vaccines have been successfully tested in pregnant cows, which transferred immunity against colibacillosis to their offspring, and the products are now available commercially. *

Using another approach to fight colibacillosis, the NBF Molecular Genetics, Inc. (U. S.) uses hybridomas to produce MAbs against the attachment antigens of the bacteria responsible for the disease. Incorporating these MAbs in milk fed to young calves within 36 hours of birth protects the animals through the period for which they are most susceptible (36]. This product is approved for use in the United States and Canada.

The development of biotechnological solutions to bacterial animal diseases, as well as viral infections, will require much basic research. Pasteurellosis (a lower respiratory tract infection in cows, sheep, and pigs) and swine dysentery (which causes annual losses of $75 million in the United States) are among the major animal bacterial infections about which more knowledge is needed before applications of biotechnology are possible. The potential for biotechnological production of new bacterial vaccines and the development of successful delivery systems is largely unexplored.

protozoan and Parasitic Infections of Animals. -Coccidiostats (compounds that prevent coccidiosis in poultry) and anthelmintics (substances that fight helminthic parasites such as roundworms, tapeworms, lung worms, and liver flukes) constitute large, rapidly expanding animal health product markets. In 1985, the global market for coccidiostats is expected to be $500 million (compared to $300 million in 1981), and the global market for anthelmintics may exceed $900 million (compared to **$450** million in 1981) (35). At present, coccidiostats and anthelmintics are synthesized by either chemical synthesis or microbial bioprocess methods. These agents, many of which have been discovered serendipitously, are commonly administered in animal feed (10).

The widespread use of coccidiostats, anthelmin - tics, and antibacterial in animal feed raises concerns about the nurturing of drug resistance among populations of micro-organisms. These risks are outlined in a 1979 OTA report entitled ***Drugs in Livestock Feed*** (30). As described in that report, the genes in bacteria that encode resistance to most drugs are located on plasmids. Resistance to drugs may be shuttled via these plasmids into pathogenic microa-oganisms such as Sal***monella.*** **Widespread use of antibacterial selects for bacteria, including Salmonella, that contain resistance genes, perpetuating drug resistance among bacteria. Thus, wide use of antibacterial in animal feed eventually may compromise the effectiveness of the same drugs in treatment of human diseases. Drug resistance among the protozoa and parasites is even less well understood than is resistance among bacteria. Such resistance is difficult to quantify but may be increasing (13,30).**

Fundamental knowledge may be gained by using rDNA technology to explore the structure and function of genes that confer resistance to drugs. MAb technology and other conventional methods may be used to isolate, purify, and better understand antigens found on parasitic cells, perhaps resulting in vaccines effective against these parasites. The increased use of vaccines would decrease the use of feed additives and presumably lessen the problems of drug resistance.

Strong needs, large market potentials, and safety considerations characterize the further development of compounds effective against protozoa and parasites that afflict animal populations. Because of the complexity of most parasitic infections, however, biotechnological solutions may not be forthcoming immediately. [n addition, the recent introduction of potent new antiparasitic feed additive compounds, such as the avermectins (which are microbially produced) (8), may lower incentives to explore new antiparasitic possibilities with biotechnology in the near term.

*These bacterial vaccines were made by replacing a "virulence gene" (a gene which encodes a protein that regulates cellular water loss and is responsible for the diarrhea) located on a plasmid with a harmless gene and "infecting" animals with bacteria containing these harmless plasmids. The bacteria continue to produce surface antigens, but they do not produce the virulence protein. The surface antigens stimulate an immune response that prevents adherence of natural virulent bacteria (18)

one serious worldwide rickettsial disease that requires urgent attention is anaplasmosis. Ana - plasmosis, which is caused by blood-borne micro-organisms transmitted to cattle by ticks, causes severe anemia and subsequent death in afflicted animals. In the United States, annual losses due to anaplasmosis are estimated to exceed tens of millions of dollars. At present, an unsatisfactory attenuated vaccine exists, and attempts to culture the micro-organism and prepare better vaccines have been only marginally effective (36).

Animal nutrition and growth promotion

Practices and products that promote animal nutrition and growth have the potential to produce direct, substantial returns on investments. Animal scientists seek better animal nutrition and feed-use efficiency in several ways, including the study of gut bacteria that participate in animal digestion, feed additives that enhance absorption of nutrients, and substances such as GHs that may directly stimulate growth and animal productivity.

Synthetic steroids and natural hormones are used widely to promote animal growth, as indicated in table 28. Furthermore, as noted above, health- and growth-promoting compounds from industrially grown micro-organisms constitute a large share of feed additives (30). Some of these compounds act by enhancing the growth of beneficial micro-organisms in the gut, others by reducing the prevalence of harmful micro-organisms and parasites throughout the gastrointestinal tract; still other compounds directly provide animal nutrition. In cases where microbial metabolic pathways and products are known, biotechnology may augment the production of com - pounds used as feed additives by increasing the production of specific microbial metabolizes. " At present, however, applications of biotechnology to the production of metabolizes largely remain unexploited **(10).**

GHs produced by rDNA technology, in contrast, currently are undergoing trials in humans and animals in efforts to demonstrate safety and effectiveness in stimulating growth. Several U.S. NBFs, including Genentech Corp. (in collaboration with Monsanto Corp.), Molecular Genetics, Inc. (for American Cyanamid), Bio-Technology General, Amgen, and Genex Corp., are producing GHs for various animal species. In addition to yielding potential commercial products, rDNA GH projects are stimulating widespread research into the nature of growth, development, and animal produc-

*The production of compounds used as feed additives is discussed in *Chapter 7: **Special t.** Chemicals and Food Additives*

Table 28.—World and U.S. Sales of Growth Promotants (millions of dollars)

	Sales								
	1979		1980		1981E[a]		1985E[a]		Compound annual growth
Products	World	U.S.	World	U.S.	World	U.S.	World	U.S.	1981-85E[a]
Hormones:									
Synovex (Syntex)	$ 14	$ 8	$ 16	$ 8	$ 19	$ 8	$ 23	$ 6	9%
MGA (Upjohn)	12	11	12	10	12	9	12	0	No change
Ralgro (IMC)	16	15	24	22	32	29	55	45	15%
Compudose (Eli Lilly)	—	—	—	—	4	—	100	50	N.A.[b]
Other:									
Rumerain (Eli Lilly)	$ 60	$ 55	$ 65	$ 55	$ 75	$ 60	$200	$125	28%
Feed	60	55	65	55	75	60	125	100	14%
Bolus	—	—	—	—	—	—	75	25	N.A.
Avoparcin ("Avotan") (American Cyanamid)	15	—	20	—	25	—	50	10	19%
Other	33	—	38	—	43	—	75	—	15 "/0
Total	$150	$ 29	$175	$ 95	$210	$106	$515	$246	250/o

[a]E = estimated.
[b]N.A. = Information not available.

SOURCE: S. J. Zimmer and R. B. Emmitt, "Industry Report: Animal Health Products Market" (New York: F. Eberstadt & Co., Inc., 1981). Modified by the Office of Technology Assessment.

tivity. The results of experiments pertaining to GHs' mode of action to date have yielded results that suggest caution. Previous observations that injections of bovine pituitary gland extracts enhance lactation in cows led to the finding that purified GHs increase milk yield by **10 to *17*** percent, without a concurrent change in feed intake (24). Other experiments with sheep and pigs have shown rapid growth following GH treatment (36). However, other evidence indicates that GHs stimulate growth and feed-use efficiency at the expense of body-fat deposition (24). Thus, critics argue, GHs may impair long-term animal health and productivity (24).

Substantial hurdles must be overcome before rDNA-produced GHs become commercially available. In addition to requiring regulatory approval, the commercial success of GHs requires an adequate drug delivery system that introduces GH slowly to animals. Oral administration of GHs, although most convenient and marketable, is an inadequate system of delivery because polypeptides such as GHs are degraded by digestive enzymes. The hormones must be made available to the body's circulation, where they can reach endocrine organs. Slow-releasing ear implants may be used as alternatives to frequent injections (injections are not amenable to most husbandry practices except those for dairy cattle), but, at present, dose requirements are too high for such implants to be practical **(21). Eli Lilly (U. S.) is developing a long-lasting bolus to be used in the rumen. Presumably, enough GH is released directly through gastrointestinal tract walls to avoid the problem of enzymatic degradation. With the development of convenient delivery systems, better field trials to investigate the efficacy of GH may result.**

„

Genetic improvement of animal breeds

Throughout the history of animal agriculture, breeders have sought to improve animal productivity by selecting animals with desirable traits for breeding. Recent increases in the understanding of animal reproductive biology and the genetic basis of traits have fostered new animal breeding technologies (31). As a result of increased knowledge due to biotechnology, the identification of genes and gene products that influence traits of productivity, vigor, and resistance to certain diseases may be possible.

In the future, animal breeding programs may be augmented by biotechnology to achieve desired changes with unprecedented speed and selectivity. Biotechnology may be used in ongoing breeding programs first to identify animals with desirable genes (e.g., genes that make the animals resistant to certain infections), and second, to transfer these genes directly into the germ line (cells that contain the genes that will be passed onto future generations) of other animals. Possible applications of biotechnology include the use of MAbs to identify and isolate gene products correlated with certain traits, the use of rDNA technology to produce large quantities of desired gene products for further study, gene transfer (micro-injection of isolated DNA into embryo cells), and implantation of the embryo cells to which genes have been transferred into surrogate mothers.

The technology of gene transfer is in its infancy. To date, it has been used only in laboratory animals, In most instances, the gene(s) to be studied is fused within a plasmid to a gene with a known "housekeeper" function required for growth. The plasmid is injected into a host cell that is deficient in the housekeeper function. Only host cells whose chromosomes incorporate the foreign DNA have the restored housekeeping activity and survive. These cells then are screened for activity of the desired gene. The GH gene has been the subject of many recent gene transfer experiments,

Thus far, gene transfer experiments in animals have increased fundamental understanding in several areas. Scientists have made great gains in preparing receptive host cells, transferring genes from one animal cell to another, and recognizing the successful recombination of foreign DNA in host chromosomes (1,6,32)33). Fundamental understanding of mechanisms of gene control in mammals has also burgeoned in recent years. Several investigations have revealed that the host tissues surrounding the cells that contain implanted genes affect expression of the foreign genes (as surrounding tissue may regulate gene

expression in normal cells) and that this "tissue-specific gene regulation" continues through successive generations (7, 12,23,25)27). Finally, gene transfer experiments have allowed the study of the expression of single genes that, with other genes, comprise traits that might be too complex for study by other methods.

Gene transfer studies may reveal much about the function of single gene products. For instance, the transfer of genes implicated in immune responses and resistance/susceptibility to disease are being studied (some of these genes encode immunological cell-surface proteins called the HLA antigens) (11). The ability to transfer such genes into foreign cells to distinguish the production and function of their products may lead to valuable knowledge about animal diseases.

In the future, gene transfer may prove to be the sole means of overcoming certain animal diseases that defy preventive vaccine technology and/or veterinary treatment. An example of such a disease is trypanosomiasis ("nagana" in cattle and "sIeeping sickness" in humans). Trypanosomiasis is caused by parasites borne in the saliva of certain insects and impedes livestock productivity throughout Africa (where the disease is transmitted by tsetse flies). Strains of cattle and sheep with resistance to trypanosomiasis (trypanotolerance) exist, and their resistance may be traceable to several distinct genes **(26,29). Gene transfer may prove useful** in better identifying these genes and selecting animals for breeding programs designed to encourage trypanotolerance in affected areas. In the future, transfer of these genes into cattle germ lines may rapidly foster widespread trypanotolerance where most other programs to control trypanosomiasis have failed. The application of knowledge gained from gene transfer experiments to animal agriculture will not be immediate, but such knowledge eventually may lead to considerable agricultural advances.

Commercial aspects of biotechnology in animal agriculture

Although field trials of several biotechnology products for animals are underway and a few products (e.g., vaccines for colibacillosis) have been approved for use, it is not yet clear to what extent biotechnologically made products will be adopted for use in animal agriculture. Most of the nascent products will require more convenient, cost-effective delivery systems, greater demonstration of effectiveness, and appropriate publicity before they are used widely.

If these challenges are successfully met, biotechnology may affect animal agriculture in numerous ways. Some novel products, such as rDNA and synthetic vaccines, in addition to lowering the costs of animal health care, may create new markets. Other products, such as diagnostic MAbs, may replace conventional diagnostic tests. At present, the animal health product markets are skewed against biologics such as vaccines in favor of pharmaceuticals such as antibiotics, mostly because biologics have not demonstrated high levels of efficacy. Until recently, commercial investment in vaccine research has been relatively small, but the wide-ranging applications of biotechnology to animal agriculture is prompting increasing amounts of investment in vaccine research. Applications of biotechnology to products for highly valued animals, such as companion animals and breeding stock, may help support substantial R&D and licensing costs associated with the first new animal drugs and biologics made using biotechnology.

Existing global markets for animal health products are shown in table 29, which differentiates major markets for nutritional products, antibacterials, and other compounds from the market for vaccines. As shown in that table, the markets for vaccines, anthelmintics, and growth promotants are expanding the most rapidly.

The companies that dominate the production of most animal health products are primarily major chemical and pharmaceutical manufacturers.* Most of these companies possess global marketing and distribution networks and undertake animal drug production as a diversification of their principal activities. As shown in table 30, animal health

*Major U.S, producers include Syntex, Pfizer Eli Lilly. 1'p;ohn SmithKline Beckman, American (: yanamid, Merck, American Home Products, Johnson & Johnson, Tech America, and Schering-Plough Major foreign producers include Burroughs-Wellcome (U.K), Rhone-Merieux (France), Hoechst AG (F R G) Bayer AG (F R G.), Connaught (Canada), Beecham (UK), Sol\ ay (Belgium) Boehringer Ingelheim (F R .(; .1, Intervet [Netherlands) and Elf Aquitaine (France).

Table 29.-Global Animal Health Product Markets

	Estimated sales, 1981 (millions of dollars)	Estimated annual growth, 1981-85
Nutritional products	*$2,500*	10-15%0
Medicinal products:		
BiologicslVaccines	1,000	20-250/o
Antibacterial	2,000	10-15%0
Anthelmintics	450	25-300/o
Ectoparasiticides	400	10-150/0
Coccidiostats	300	15-200/o
Growth promotants	200	24-300/o
Other	650	15-200/o
Subtotal	5,000	15-200/o
Total	$7,500	15-200/o

SOURCE: S. J. Zimmer and R. B. Emmitt, "Industry Report: Animal Health Products Market" (New York: F. Eberstadt & Co., Inc., 19S1).

Table 30.—U.S. Producers of Animal Health Products

	Estimated animal health sales, 1981 (millions of dollars)	Percent of corporate sales	Percent of corporate operating income	Estimated animal health sales annual growth, 1981-85
Pfizer	$440	13 "/0	130!0	100/0
Eli Lilly	365	130/0	150!0	200/0
American Cyanamid	265	7%0	**7"/0**	**11%0**
Merck	200	70/0	7"/0	270/o
SmithKline	155	70!0	50/0	1 70/0
Upjohn	134	70!0	7"/0	110/0
Syntex	83	120/0	N.A.a	110/0

[a]N A. = Information not available.

SOURCE S. J Zimmer and R B. Emmitt, "Industry Report Animal Health Products Market" (New York: F, Eberstadt & Co., Inc , 1981)

product sales by the U.S. companies that produce such products constitute a fairly low percentage (an average of 11 percent) of the companies' total sales. Investments in animal-related biotechnology R&D in those companies probably average about the same or less than the investments by the leading NBFs that are applying biotechnology to animal agriculture (22).

As noted in ***Chapter 4: Firms Commercializing Biotechnology,*** most major pharmaceutical and veterinary medicine companies are investing in biotechnology R&D, but there is some question as to their motivation for producing new products for large, established animal health care markets. Such markets include those for antibiotics, anthelmintics, and coccidiostats. Established companies with existing lines of conventionally made, widely marketed animal health products may have strong interests in maintaining these products. In many cases, therefore, their primary interests do not lie in R&D to produce new animal health products. As described earlier, applications of biotechnology to the production of animal products involve a substantial investment in basic research. In some cases, healthy sales of conventionally made products may dissuade a company from pursuing basic research that could lead to the development of a competing product. In other cases, corporate developers may choose to pursue human pharmaceutical innovations of new biotechnology, rather than applications of biotechnology to animal health. Because of these considerations, innovation and new product development in animal agriculture might be slowed.

Innovation in smaller product market areas, such as animal vaccines and diagnostic products, however, is widespread, New or replacement animal vaccines are among the most promising applications of biotechnology, as are MAb-based diagnostic products, Much of the innovation in

developing these products is attributable to NBFs in the United States.

At present, the extent to which biotechnology will be used for the development of animal vaccines is uncertain. Most individual vaccine markets are relatively small—as shown in table 31, most U.S. vaccine markets for animals range from $5 million to $10 million per year—and sales of a single vaccine line would probably be insufficient to sustain a company. Therefore, most companies must market a broad range of vaccines to be competitive. The practice of maintaining a diverse selection of these products may facilitate the development of vaccines for diseases that alone might be unprofitable, such as diseases endemic to developing countries (16). Ultimately, the application of biotechnology to animal vaccine development depends on technical feasibility and the ability of vaccine developers (currently, mostly NBFs) to obtain funding for further work.

In addition to improving vaccines for a broad range of animal diseases, biotechnology may shift the sites of vaccine production from several large foreign producers (e.g., Rhone-Merieux (France) and Burroughs-Wellcome (U.K.))* to smaller U.S. producers. Currently, as shown in table 32, three foreign manufacturers control approximately 25 percent of the U.S. veterinary vaccine market (which accounts for one-fourth of the world's yearly $1 billion veterinary vaccine market). With the successful development of subunit veterinary vaccines by U.S. NBFs, competition may result in a redistribution of worldwide vaccine production. Collaborative arrangements between NBFs and local producers for the development of safe subunit vaccines effective against local strains of animal diseases may increase in the future.

Conclusion

The use of biotechnology to improve animal feed, nutrition, and health promise to improve production of food from animals. MAb-based diagnostic products exemplify this promise. Other products, such as new vaccines, may face technical problems of dosage, formulation, and delivery before they are suitable to animal husbandry practices. Until these problems successfully are resolved, the impact of biotechnology on improving animal productivity will not be realized. Applications of biotechnology such as gene transfer experiments and investigations into the nature of growth using rDNA-produced GHs currently serve to increase basic knowledge about animal biology.

Table 31.—Sales of Major U.S. Animal Vaccine Products, 1981 (millions of dollars)

	Sales
Cattle products:	
Clostridium	$ 16.0
Infectious bovine rhinotracheitis and bovine leukosis virus	13.0
Leptospirosis and combinations	6.0
Vibriosis and combinations	3.0
Swine products:	
Atrophic rhinitis (Bordetella)	$ 6.0
Pseudorabies	5.0
Erysipelas	3.5
Pet products:	
3-way feline virus disease	$ 4.5
Rabies	12.0
Canine parvovirus and combinations	9.0
Poultry products:	
Marek's disease	$ 12.0
Newcastle disease and combinations	9.0

SOURCE: S. J. Zimmer, "The Impacts of Applied Genetics in Animal Agriculture," contract report prepared for the Office of Technology Assessment, August 1982.

Table 32.—Major Producers of Animal Vaccines Sold in the United States

Company	1981 sales (millions of dollars)	Market share
Norden (SmithKline) (U.S.)	$40	27%
Philips-Roxane (Boehringer Ingelheim) (F.R.G.)	18	12
Fort Dodge (American Home Products) (U.S.)	14.5	10
Beecham (U.K.)	11	7
Jensen Salsbery (Wellcome) (U.K.)	9	6
Dellen (TechAmerica) (U.S.)	8.6	6
Pitman-Moore (Johnson & Johnson) (U.S.)	1.8	1
Syntex Agribusiness (U.S.)	1.5	1

SOURCE: S. J. Zimmer, "The Impacts of Applied Genetics in Animal Agriculture," contract report prepared for the Office of Technology Assessment, August 1982.

*Rhone-Merieux and Wellcome command the international markets for rabies and FMD vaccines. Together these two vaccines comprise 30 to 35 percent of global animal vaccine sales. Other leading FMD vaccine producers are Bayer (F.R.G.), Pfizer (U.S.), Hoechst (F.R.G), and Rosenbusch (Argentina). State agencies serve about one-half of the rabies market, and Rhone-Merieux, Wellcome, and Connaught (Canada) dominate the rest.

Plant agriculture

There are hundreds of forms of crop improvement whose purpose generally falls into one of three categories. The first is to increase crop yields by increasing resistances to pests or environmental conditions such as drought or soil salinity or by developing more productive plants, The second is to improve crop quality by enhancing such features as nutritional value, flavor, or processability. The third is to reduce agricultural production costs by reducing a crop's dependence on chemicals or by making harvesting easier (55, 56).

During the last century, plant breeders have been efficiently and successfully addressing all of these goals. The use of new biotechnology in crop improvement, as in other areas, is not a new beginning, but an extension of previously evolved skills. New biotechnology alone will not produce better crop plants, but combined with knowledge from other plant science and microbiological dis. ciplines, biotechnology will develop techniques that could be very powerful in improving agriculturally important crops. Thus, the greatest advances in crop improvement are likely to be made using an interdisciplinary approach.

The genetic manipulation and modification of plants presents some special challenges. Most molecular genetics to date has been done with simple unicellular organisms and, to a lesser extent, with laboratory animals. The application of molecular genetics to plants is relatively more recent and consequently at an earlier stage of technical development. Furthermore, there are fewer studies of the physiology and biochemistry of plants than there are studies of these aspects of animals. The recent application of the new techniques of molecular biology to plants has produced astoundingly rapid results, however, and these techniques are sure to have an impact on crop improvement in the next several years.

Of the several hundred domesticated plants in the world today, only about 30 are of great economic significance. Of these, eight domesticated grains, including rice, corn, and wheat, produce most of the calories and protein consumed by humans and agriculturally important animals. The legumes, which include soybeans, represent the second most common source of food for human and animal consumption. There are two philosophies, which are not incompatible, with regard to improving crop plants. One is that there should be diversification of crop plants and attention given to the domestication and breeding of new **major** crop plants. Another philosophy is that plant breeding, tissue culture, and biotechnology efforts should be devoted to the most successful crop plants. The genetic diversity of some of the world's current crop plants is not great. Consequently, even if the major crop plants are the focus of research efforts, some genetic material from exotic sources may be required to effect the desired improvements. In any case, the techniques discussed here are equally applicable to the improvement of both common and exotic species,

Research on plants has shown that the genetic organization of plants exhibits striking similarity to that of animals. The universal genetic code is used, and most genes contain intervening sequences and are surrounded by very similar regulatory sequences. Unlike animals, however, plants have a characteristic called totipotency that, for many species, indicates the potential for regeneration of a single cell into a complete plant. Because plants have this totipotent characteristic, certain genetic manipulations can be done in cell culture, and, after selection of cells with the appropriate qualities, the cells can be regenerated into parental plants (for breeding programs). Adjusting the laboratory variables to achieve regeneration from single cells is evolving from an art to a science and has yet to be accomplished consistently for the principle cereal grains (monocots *), but regeneration research is proceeding at a rapid rate. It is likely that many important crop plants will be able to be regenerated from single cells in the next few years.

There are several potential applications of new biotechnology for plants that may help in the im-

*Early in the evolutionary history of flowering plants, two main types of plants, monocots and dicots, diverged. Cereal grains (corn, wheat, rye, barley, rice, etc.) are monocots, whereas legumes (soybeans, etc) are dicots

Phofo Credit Dean Eflgler Agrfgenef~c5 Corp

Freshly isolated plant protoplasts

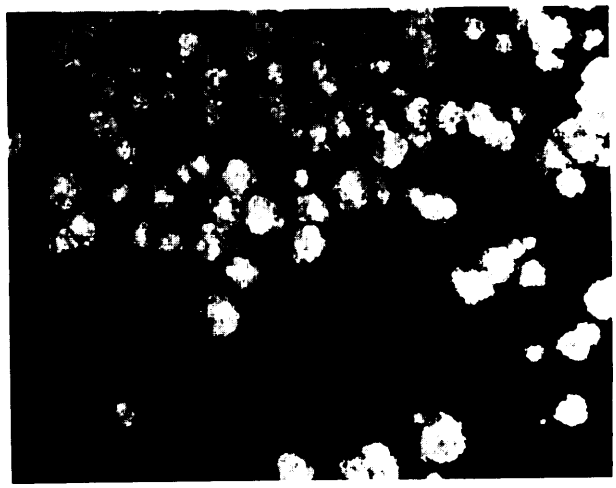

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Photo Credit" Dean Engler, Agrigeneflcs Corp

Plant shoots arising from protoplast-derived calli

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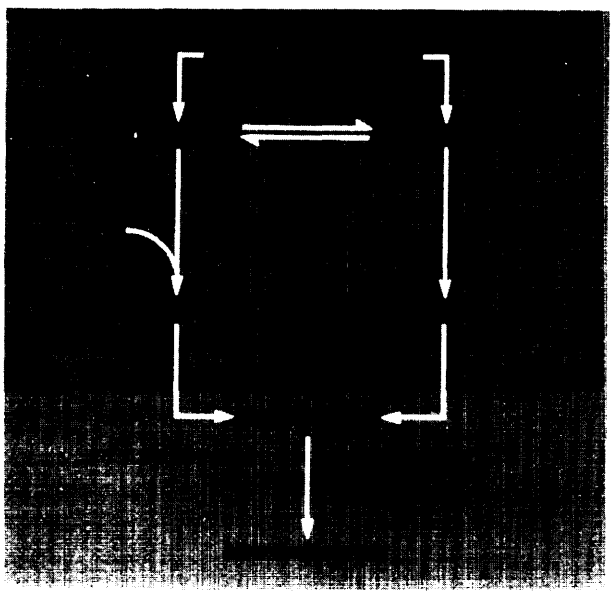
provement of crop species, as shown in figure 16. New technologies for testing for the presence or absence of traits, for example, will save years of plant breeding time. Many applications to plant agriculture will be in the regulation of endogenous genes, and other improvements will be made using techniques such as the following, which transfer DNA from one species to another:

- The fusion of cells from two different plant species can be used to overcome species hybridization barriers. In order to be useful, the resulting cell fusion product must be regenerated to form a whole plant. To date, regenerated plants have only resulted from fusions between closely related genera (62). The regenerated plants are selected to express beneficial characteristics of both parents (94). As yet, no economically important variety has been produced using this method (62).
- Transferring subcellular organelles such as nuclei or chloroplasts from one plant species to another can be accomplished by a variety of techniques. One of these, liposome transfer, involves surrounding the organelle with a lipid membrane. Because chloroplasts carry many of the genes important in photosynthesis, liposome transfer may be instrumental in improving photosynthetic efficiency.
- Vector-mediated DNA transfer (and microinjection of DNA) is the most specific, and potentially the most versatile, of the genetic manipulation techniques. Recently, foreign plant genes have been inserted and expressed in plants.

Figure 16.–Steps To Create a New Variety of Plant by Using Biotechnology



SOURCE. Office of Technology Assessment

Recent advances in the methods of plant cell culture and the techniques for introducing DNA from one plant species to another are discussed in ***Box C.—Methodology Important in Plant Agriculture.*** The applications of these methods to specific problems in plant agriculture, such as disease resistance, photosynthetic efficiency, and nitrogen fixation and the commercial aspects of biotechnology in plant agriculture are discussed in the sections that follow.

Improvement of specific plant characteristics

Greater crop yields or a reduction in the cost of crop production would be possible if plants were resistant to disease and certain environmental factors and contained a larger amount of higher quality product. In the United States, there is great research interest in crop resistance and crop quality improvement in academic, Federal, and industrial laboratories. Unlike most other plant traits, some resistances and specific improvements may be accomplished with one or a few gene modifications. This area, therefore, is probably the most active area of industrial research, and it is likely that considerable research progress in this area will be made in the next 5 to 10 years.

PLANT RESISTANCE FACTORS

Disease and environmental resistances are important to most crops in most areas of the world. Important plant resistances are shown in table 33. Productivity losses often can be attributed to the lack of resistance to one or more factors (see tables 34 and 35). Thus, the study of resistances could lead to greatly improved productivity and an increased realization of genetic potential (42).

Numerous single gene resistance factors are known in higher plants. The most common re-

Box C—Biotechnology Improvements in Plant Agriculture

Methods of Plant Cell Culture

Plant cell culture is important commercially (for [illegible] species of plants can be cultured and regenerated, allowing many identical plants, [illegible] be grown. For example, cultured plant cells are used for selection of [illegible] for yields in potato crops to be greatly reduced by several potato viruses. [illegible] plants are now obtained from regenerated cell-cultured potatoes, and, as a consequence, [illegible] plants has increased substantially (98).

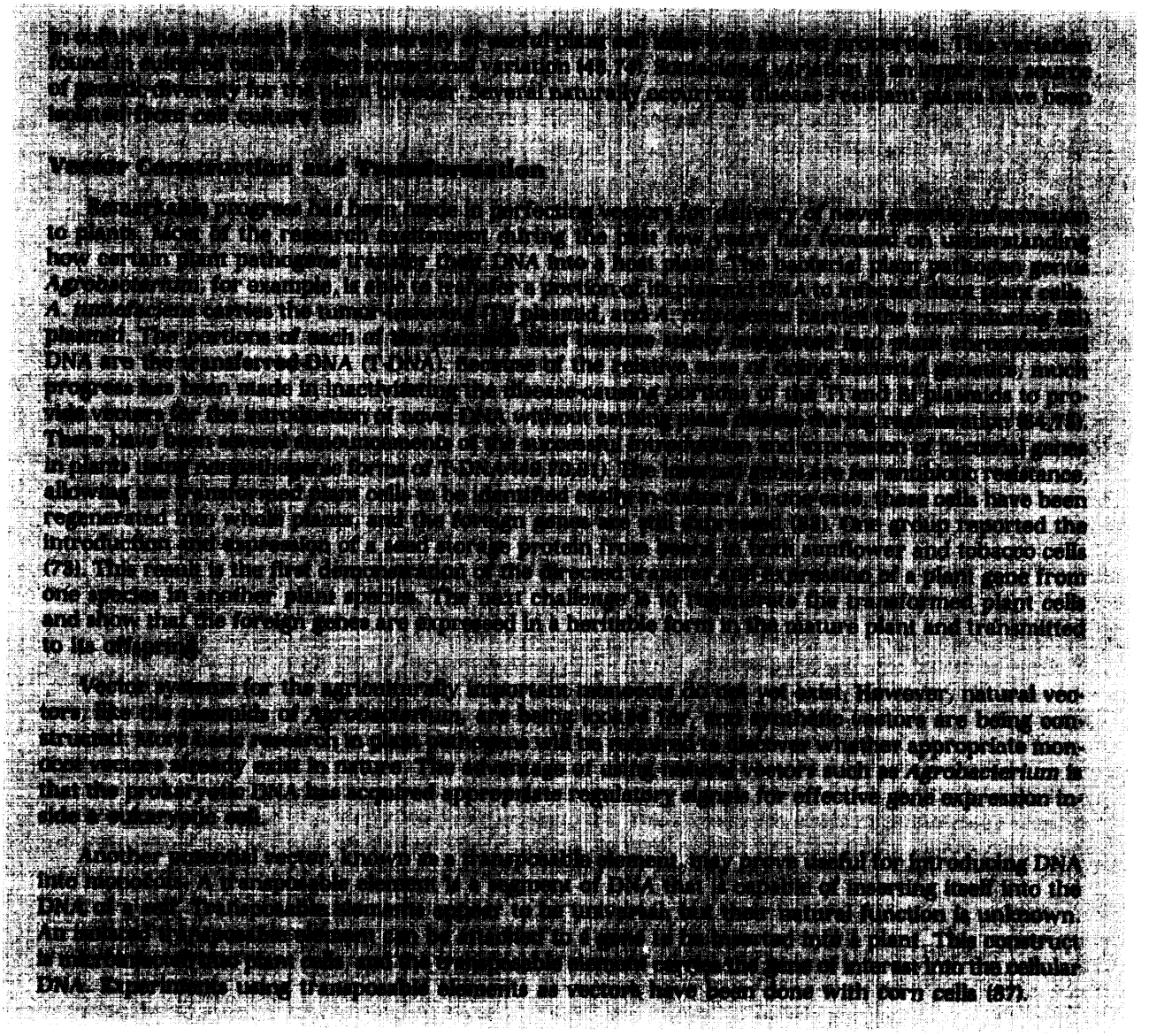
In the research laboratory, plant cell culture [illegible] molecular genetics and plant breeding. Most schemes involving the transfer of [illegible] require that cells from the recipient plant be cultured, although it should be noted that [illegible] experiments can now be done by the direct manipulation of pollen or embryos, [illegible] the necessity for regeneration. If single plant cells or embryos can be cultured, [illegible] number of cells in any experiment can be large, considering that the [illegible] of plant cells can be studied in a small laboratory. [illegible] plants were to be used in an experiment, areas of greenhouse or field [illegible] The development of cell culture conditions is far from routine, and even though [illegible] of plant cell culture are notable, there are still only a limited number of species [illegible] routinely (53).

Plant cells are useful to geneticists, but to be [illegible] naturally, whole plants must be able to be regenerated from those cells. Regeneration is [illegible] by placing the cells under appropriate conditions of light, temperature, nutrition, and growth [illegible] early stage of regeneration is the formation of an undifferentiated clump [illegible] from the callus arise the differentiated cells of roots and stems. Alternatively, [illegible] can be induced from the callus. The crop species for which [illegible] routinely include asparagus, rapeseed, [illegible], potatoes, and tobacco (64). Currently, [illegible] corn, wheat, and [illegible] to routine method published for [illegible] any inherent differences in these species, [illegible] with only a few model systems. Now that direct genetic manipulations can be [illegible] important plants, there is renewed interest in developing [illegible]

One recent [illegible] calli while retaining cell viability. Another [illegible] to preserve materials (101). One [illegible] embryos of rare mutants for [illegible] of the methods of freezing [illegible] tropical trees, for example—until ecological niche [illegible] has been solved. Also, a seed embryo banks might replace or supplement some seed storage [illegible] The initial applications of these methods will simplify laboratory maintenance of cell cultures.

The ultimate [illegible] molecular biology is to create improved [illegible] of plant cells where the cells are [illegible] the desired trait, a high percentage [illegible] reproductive plant. Although many [illegible] have been demonstrated, the outlines [illegible] innovations could provide suitable cell culture and regeneration protocols for many important crop species.

One important fact recognized in the past few years is that there sometimes is extreme variation in the phenotype and genotype of cells in tissue culture. In the past, mutants were sought after mutagenesis treatment. Beginning with work on sugarcane, however, the variability uncovered or created



sistance genes are those that confer decreased susceptibility to disease (54,71,79). In maize, for example, there are resistance genes to several diseases such as northern corn-leaf blight (s1). Because most of the single gene resistance factors confer resistance to a single pathogenic organism, it is thought that a single characteristic of the host and pathogen determine the outcome of an infection.

Most of the existing disease-resistance genes have been introduced into economically important lines of interbreeding plant species by traditional plant breeding. Currently, however, there is interest in cloning disease-resistance genes from plants in order to study the nature of resistance and to determine the possibility of transferring resistance factors among species that do not normally interbreed. It is not known in most cases

Table 33.—Plant Resistances of Economic Value

Resistance to:	Relevance in United States
Disease	All crops
Saline	Irrigated soils, particularly in California and Southwest
Alkaline earth metals	Southeastern United States and West
Anaerobic soil conditions	Areas subject to flooding
Drought	All crops
Herbicides	All crops
Pesticides	All crops
Soil pH	Low pH on acid mine tailings and soil affected by acid rain; high pH on most Western soils

SOURCE Office of Technology Assessment

Table 34.—U. S. Soils With Environmental Limitations

Environmental limitation	Percentage of U. S. soil affected
Drought	25.30/o
Shallowness	19.6
Cold	16.5
Wet	15.7
Saline or no soil	4.5
Alkaline salts	2.9
Other	3.4
None	12.1

SOURCE: J S Boyer, "Plant Productivity and Environment," Sclefrce 218443-448, 1982

Table 35.-Distribution of Insurance Indemnities From Crop Losses in the United States From 1939 to 1978

Cause of crop loss	Proportion of payments (%)
Drought	40.80/o
Excess water	16.4
Cold	13.8
Hail	11.3
Wind	7.0
Insect	4.5
Disease	; : ;
Flood	
Other	1.5

SOURCE" J. S Boyer, "Plant Productivity and Environment," *Science* 218443-448, 1982

what the disease-resistance genes in plants actually do to plant metabolism or structure. By understanding how the products of plant disease-resistance genes work, better screening programs for enhanced resistance can be designed. Environmentally, it is desirable to develop pest-resistant plants, because such plants would reduce the need for spraying crops with pesticide * chemicals, and disease control would be more effective. It should be kept in mind, however, that much of the agricultural research effort is being made by the agricultural chemical industry, and this industry may see the early opportunity of developing pesticide-resistant plants rather than undertaking the longer term effort of developing pest-resistant plants.

Resistance to environmental conditions probably depends on both single and multigenic inheritance. These traits, as well as disease resistance, can be selected for in tissue culture. If analogs of the disease or detrimental environment conditions can be applied to plant cells in culture, the entire procedure can take place in a few test tubes or petri dishes in a laboratory setting. Millions of individual cells can be treated simultaneously and then examined for survivors. Stepwise selections under gradually more stringent conditions (e.g., a *gradual increase in* the salinity of the medium) are accomplished readily.

Some of the traits that could be selected in tissue culture are listed in table 33. Many of the traits are resistance factors that confer protection against disease and salinity. In selection schemes for these factors, the test organism is exposed either to normally lethal doses of the toxins produced by a disease organism or to high doses of salt (to mimic salinity), and the surviving cells are identified by their growth under these normally toxic conditions. This protocol holds great promise for identifying rare cells that have spontaneously acquired a novel resistance. Somaclonal variation probablv supplies much of the variation seen in tissue culture (see box C) (47).

A rate-limiting step in applying selection techniques more widely is the present inability to regenerate major cereal and legume crops from individual cells or small cell clumps on a routine basis. Furthermore, some of the traits selected in tissue culture for resistance to a specific factor may not be manifested in the whole plant, because it is possible for cells to develop nongenet -

‘,4 pesticide is an agent (hat prevents the growth or propagation of deleterious organisms, including weeds and insects Both herbicides and insecticides are pesticides.

ic adaptations. Consequently, numerous regenerated plants will be required to determine if a particular selection procedure yields whole plants with important agronomic traits. on the other hand, pollen or embryo manipulation may circumvent some of these problems.

Genetic manipulations can make plants resistant to chemicals or can enhance their response to chemicals. These traits are of particular importance to the agricultural chemical industry. For instance, various plant growth regulators are produced by this industry. These chemicals can affect many stages of the growth or reproduction activities of plants to give a crop with increased yield. Enhanced response to these chemicals allows the crop to be grown at a lower cost.

Producing herbicide-resistant plants can have definite benefits, especially in crop rotation. For instance, corn is naturally resistant to triazine herbicides, whereas soybeans are not. Occasionally, soybeans do not grow well in a field the year after triazine-sprayed corn was grown there. In this case, one solution would be to introduce triazine resistance into soybeans. This particular resistance is due to a modified protein in the chloroplast membrane. Therefore, resistance between dissimilar species could be transferred by protoplasm fusion or liposome-mediated chloroplast transfer (38,66). It should be noted, though, that increased use of agricultural chemicals could have serious environmental consequences.

PRIMARY PLANT PRODUCTS

The largest research effort in the modification of plant products using biotechnology is concentrated on the improvement of seeds and seed proteins. Seeds serve a dual role in agriculture. They are the major source of food for people and animals and represent an easily stored form of plant material, and they are also the material for propagating the next plant generation. The storage materials of seeds contain all of the materials necessary to nourish a plant, because each seed must support the initial phases of germinlation and seedling establishment until the plant is self-sufficient. During domestication, various crops have undergone an enormous exaggeration of the normal storage reserves. Today, far more material is stored in agricultural crop seeds than in the seeds of wild relatives; sometimes the increase is as much as tenfold (68).

Although the agronomic (applied) research effort is devoted primarily to increasing the amount of seeds and seed protein, current basic research efforts are devoted both to increasing the quality of the stored materials and to exploring plant gene structure. Because plants are capable of synthesizing all of the amino acids required for protein synthesis from simple carbon- and nitrogen-containing precursor molecules, the exact amino acid composition of the stored protein in seeds may not matter to a plant, and seed proteins often have an unbalanced amino acid composition. Because humans and most animals are unable to synthesize eight amino acids (the essential amino acids), the composition of ingested protein matters very much in their nutrition,

Much is known about the structure of the storage-protein products and the genes encoding these proteins in the major crop plants. In all cases studied so far, the storage-protein genes are found in small gene families with 3 to 30 members. Typically, a few genes of the multigene family contribute a significant fraction of the total protein, There is not much genetic variation among the seed storage-protein genes of a given species, although this low variation might be due to the limited diversity of the varieties currently studied, These crops may have lost much of the original diversity present in the progenitor species during the intensive plant breeding activities that have occurred throughout history.

DNA clones of storage proteins are available from several crop species: soybean, garden bean, corn, wheat, and other less significant crop plants. Changes in these genes can be made readily in vitro to improve the balance of amino acids in the protein. The difficult part is reintroducing the altered storage-protein gene back into the crop plant and ensuring that this novel gene is expressed appropriately. Most storage proteins are present only in seeds. Retention of this tissue specificity is important; storage proteins' presence in other plant cells may be detrimental. Another important consideration is that the storage-protein genes are found in families. Introduction of a new gene may change only a fraction of the total protein produced. To modify the overall amino

acid composition, several genes may have to be introduced or the natural genes deleted and replaced by novel genes. In some crops such as corn, there are mutations that reduce the production of zein, the storage protein. These mutant genes can be used to reduce the zein concentration, thus allowing an introduced gene to have a *greater* impact on overall amino acid composition.'

An alternative to the modification of existing crop species' genes is the introduction of completely novel genes isolated from other organisms. Genes whose products are very rich in the amino acids that are deficient in a particular seed type could be introduced to increase the concentration of specific amino acids. One promising example of this type is the storage protein of the Brazil nut **(97).** This protein is composed of **25** percent sulfur-containing amino acids (methionine and cysteine). Legume seed protein usually is deficient in these amino acids. Introduction of a few copies of the Brazil nut storage-protein gene into legume species might overcome the sulfur amino acid deficiency. Proteins of unusual composition may offer the quickest method of preparing a gene to complement deficiencies in major crop storage proteins.

SECONDARY COMPOuNDS FROM PLANTS*

Table 36 lists some of the desirable secondary products from plants. Very little research has been done on the tissue culture production of these compounds, yet it should be possible to produce important high-value plant products using culture systems instead of gathering plants from nature. Cell culture offers the advantages of reproducibility and control over production where seasonal variations, weather changes, or disease are not problems (40,57,95). On the other hand, a difficulty in the production of some products is maintaining the plant cell culture in a differentiated state such that compound production occurs.

Biotechnology offers many opportunities for the production of secondary plant compounds. The transfer of the plant metabolic pathway for a compound to a bacterial or fungal cell, for instance, could offer an opportunity for providing a steady supply of these compounds, although much more knowledge concerning the genetics and biochemistry of the pathways that produce these compounds is necessary. Another possibility is identifying and modifying the gene coding for the enzyme responsible for the rate-limiting step in product production. Overproduction of the products could result from the plants being grown in culture or in the field.

There is little current U.S. research effort to improve the yield of secondary plant compounds from cultured cells or whole plants. The Federal Republic of Germany, Canada, India, and Japan, on the other hand, have large research programs, as measured by the number of papers presented at the 1982 International Congress of Plant Tissue and Cell Culture (58). Japan, for instance, has scaled up the growth of tobacco cells to 7,000 liters, and researchers at the University of British Columbia are growing 100 liter batches of Mada-

Table 36.—Examples of Secondary Plant Products of Economic Value

Agricuitural chemicais:
Pyrethrins
Rotenone
Nicotine
Allelopathic compounds
Antibiotics against soil microbes
Pharmaceutical drugs:
Codeine
Morphine
Steroids
Cardiac glycosides
Alkaloids
Reserpine
Retinoic acid
Caffeine
Cannabinoids
Antitumor compounds
FiavorIngs and saits:
Licorice
Coumarin
Coiorings and pigments:
Anthocyanins and betacyanins
Carotenoids
industrial intermediates:
Latex
Lignin
Dye bases
Steroid and alkaloids products

SOURCE Off Ice of Technology Assessment, adapted from E A Bell and B V Charlwood (ads.) *Secondary Plant Products (New* York" Springer-Verlag, 1980).

*This topic was covered by a recent OTA workshop entitled "Plants: The Potent ial for Extracting Protein, Medicines, and other Useful Chemicals" (99)

gascar periwinkle cells in order to isolate anticancer compounds (58). In fact, the Japanese Government is spending $150 million over 10 years for research on obtaining secondary compounds from plants. It is argued by some, though, that plant cell culture for producing secondary products is necessary only when good farm land is not abundant (65).

PLANT GROWTH RATE

The rate at which plants grow can limit both the amount of harvestable biomass (food, fiber, secondary products) and the length of time between planting and harvesting. Traditional plant breeding has been quite successful in modifying and improving plants to respond to modern agricultural practices of herbicide, pesticide, irrigation, cultivation, and high-fertilizer application. These breeding programs have established that there is no single gene for yield. On the other hand, much is known about the genetics of harvestable products such as seed size. Additionally, there are single gene mutants, such as that for gibberellic acid, that can affect plant growth dramatically. Increased understanding of these areas of genetics may have an impact on this area of plant biology. For instance, a plant can be imagined that had a decreased amount of total biomass but an increased amount of harvestable product.

PHOTOSYNTHETIC EFFICIENCY

Photosynthesis is the basis for most life on Earth. Higher green plants, algae, and some bacteria can utilize the energy in sunlight to split water molecules; in this process, energy is generated and utilized to combine atmospheric carbon dioxide (CO,) into an organic form as well as to drive other energy requiring processes of plants. A byproduct of this reaction is molecular oxygen (O_z). Thus, photosynthesis is not only the ultimate source of fixed carbon we use as food and fiber, but also of the oxygen we breathe.

Because photosynthesis is so important to food production, much research has focused on the mechanism of photosynthetic action. The photosynthetic system is very complex, combining enzymatic activities, key roles played by cellular organelles, and plant anatomy as well as environ-mental factors such as light, water, and temperature. Many proposals have been made to improve the efficiency of this system by genetic manipulation.

The critical step of the photosynthetic CO_z fixation cycle is catalyzed by the enzyme ribulose bisphosphate carboxylase (RuBPCase), probably the most abundant protein on Earth. This enqyme is sequestered in chloroplasts, the cellular organelles where photosynthesis occurs. It is a complex molecule synthesized from both chloroplast genes and nuclear genes (50,51).

When photosynthesis originated, the Earth's atmosphere is postulated to have been nearly devoid of oxygen. The oxygen we have today is a byproduct of photosynthesis, and oxygen comprises about 20 percent of our atmosphere. RuBPCase initially evolved in a low oxygen atmosphere but now must fix CO. with a large excess of 0. present. RuBPCase can utilize this 0, in what appears to be a nonproductive enzymatic reaction. This process is called photorespiration and results in a net loss of fixed CO_z (45). Photorespiration can decrease crop yields by as much as 50 percent (82). It is ironic that RuBPCase activity over the past millions of years has produced the O_z that now decreases the efficiency of photosynthesis. On the other hand, it has been postulated that the ubiquitous and continued presence of photorespiratory activity implies some natural selection advantage **(61).**

Suggestions have been made for modifying RuBPCase or other enzymes involved in the photosynthetic system. For instance, genetic manipulations that would increase the affinity of RuBPCase for CO_z or decrease its affinity for O_z could substantially increase net CO_z fixation. It has yet to be determined what effects these changes would have on the survivability of plants.

In addition to manipulating the enzymatic system, changing the plant's anatomy, such as the types of cells in leaves, might be possible. Several groups of higher plants have increased rates of CO_z fixation that correlate with modified anatomy and physiology. Very little is known about the genetic control of leaf and cellular anatomical development, so near-term success in modifying these aspects of plant anatomy is unlikely.

Genetic manipulations to increase photosynthetic efficiency, and consequently food production, are very difficult now because of the complexity of the system. It will be several years before rDNA technology will aid in producing agriculturally important plants with increased inherent photosynthetic efficiency.

PLANT-PRODUCED PESTICIDES

Some species of plants are highly resistant to potentially damaging insects. Although not very much is understood about this phenomenon, it appears that certain plants can produce compounds that are toxic to specific species of insects **Or that interfere w'ith the insects' normal reproductive or growth functions (86). An African plant, for example, produces a compound that interferes with a particular caterpillar's molting, and, as a result, the insect cannot eat (48). other plants are known that produce chemicals that cause potentially harmful insects to avoid those plants for feeding or egg laying (59). The specificity of these plant-produced insecticides and nonpreference chemicals allows the control of pests while permitting potentially useful insects to survive. Many applied chemical pesticides do not have this specificity. 1t may be feasible soon to clone and transfer the genes that code for these naturally occurring chemicals, allowing them to be expressed in other plants. The result of these gene transfers could be to reduce greatly the amount of agricultural chemicals needed and, hence, the cost of production.**

Investigations into chemicals released by some plants that adversely affect neighboring plants is receiving an increased amount of attention ($10). These herbicides, known as allelopathic chemicals, may influence another plant directly or may act by inhibiting the micro~organisms normally associated with that plant. Allelopathic chemicals consist of a wide variety of chemical types, and their actions range from inhibiting cell division to protein synthesis to photosynthesis. Much more still is to be learned about these naturally occurring chemicals, including the factors influencing their production and how best to use them agriculturally. A goal of biotechnology is to identify the genes responsible for the synthesis and release of the plant pesticides and to transfer them to nonresistant plants. Biotechnology also could aid in the understanding of their production and possibly help develop their production in controlled laboratory culture systems.

Uses of micro-organisms for crop improvement

Applications of biotechnology in the area of crop improvement include genetic manipulations of micro-organisms that interact with plants in nitrogen fixation, for example, or that produce substances such as insecticides of potential benefit to plants. These applications are discussed further below.

NITROGEN FIXATION

Plants have a universal need for metabolically usable nitrogen in the form of ammonia (NH), which can originate either from the air or from applied ammonia fertilizer. Biological nitrogen fixation, the process by which living systems convert nitrogen gas in air to NH, is catalyzed in living systems by the enzyme nitrogenase. Nitrogenase, and consequently the capacity to fix nitrogen, is found only in prokaryotes, either bacteria or blue-green algae. Some nitrogen-fixing prokary - otes are free-living and can be either anaerobic or aerobic; other prokaryotes fix nitrogen only when they coexist symbiotically with a higher plant host. The application of biotechnology to nitrogen fixation may result in more efficient prokaryotic nitrogen fixation or the transfer of nitrogen-fixing ability to plants themselves.

Nitrogen-fixing prokaryotes share some common physiologic features. First, nitrogen fixation typically does not occur in cells already supplied with usable nitrogen. Second, nitrogenase is oxygen-sensitive, so all nitrogen-fixing organisms have mechanisms for limiting oxygen. Third, NH,, which is toxic at high concentration, must be converted readily into organic nitrogen,

Biological nitrogen fixation is energy intensive (84,88,93), and in plant-microbe associations, this energy is derived from the plant. Estimating the energy expenditures for biological nitrogen fixation is difficult, and few reliable numbers are available. The energy cost of nitrogen fixation is

an appropriate concern, but this cost should be compared with the true cost of nitrogen nutrition in field-grown plants (i.e., the cost of chemical fertilizer synthesis and other biological costs to the plant).

It may be possible to decrease the energy required for nitrogen fixation by 30 to 50 percent by preventing the evolution of hydrogen during nitrogen fixation. Some bacteria have a set of genes that allow for hydrogen recycling. These genes have been cloned and inserted into less efficient nitrogen-fixing bacteria. The recipient bacteria showed increased nitrogen-fixing efficiency (37).

Agriculturally important nitrogen-fixation systems discussed below are nonlegume nitrogen fixation and symbiotic nitrogen fixation in legumes.

Nonlegume Nitrogen Fixation.—Nitrogen fixation is performed by several groups of bacteria and blue-green algae that live free in soil or in aquatic habitats. The best studied nitrogen-fixing bacterium is the free-living ***Klebsiella pneumonia,*** which can easily be grown in the laboratory. * The gene complex coding for the nitrogen-fixing function in ***Klebsiel]a pneumonia*** is comprised of 17 genes, and the regulation and activities of these genes now are being studied extensively. Still, the nitrogen-fixing function is extremely complex and not well understood.

Algae have been used to fix nitrogen in Asian rice paddies for many years. Recently, research has produced strains of algae that could be used in soil to fix nitrogen for domestic crops. Algae are inexpensive compared to nitrogen fertilizer, and because they release nitrogen slowly into the soil, algae bypass the problem of nitrogen leaching (60). Furthermore, algae are being considered the botanical equivalent of yeast for genetic manipulation, and vector systems for algae transformation are in development (41).

Symbiotic Nitrogen Fixation in Legumes.—The legume-llhizobium symbiosis is the most agriculturally significant biological source of fixed nitrogen. Both grain and forage legumes have large amounts of nitrogen fixed by ***Rhizobium.*** Recent work on ***legume-Rhizobium*** symbiosis has focused on several areas, including the determination of energy costs, pathways of nitrogen assimilation and transport, the biochemistry of symbiotic nodule development, and the genetics of the bacterial partner.

Symbiotic nitrogen fixation can be a significant source of nitrogen nutrition for legume crops, but its practical application can be limited by several sets of factors, some environmental, others intrinsic to the plant-bacterial partners. Soil conditions and environmental levels of fixed nitrogen have significant effects on rhizobial survival, nodule formation, and levels of nitrogen fixation. One crucial area, poorly understood at present, is the role played in symbiotic nitrogen fixation of soil micro-organisms other than ***Rhizobium.*** Understanding nodule formation in detail will help explain environmental effects on infection that may relate to competitiveness and effectiveness of various ***Rhizobium*** inocula. In addition, an understanding of why legumes, and not other plants, can nodulate would be essential for attempting to extend host range.

Another nitrogen-fixing micro-organism, the actinomycete ***Frankia,*** is of interest because it nodulates a number of unrelated plant genera. This ability suggests a simpler genetic symbiosis than that of ***Rhizobium*** and legumes. If this is true, it may be easier to extend genetically the host range of the symbiotic relationship of ***Frankia*** than to extend that of ***Rhizobium (41).***

Specific host proteins are produced in nodules. One of these is leghemoglobin, which controls the oxygen content of the infected nodule cells. This protein is produced in high quantities in nodules. Two research groups have cloned the genes for soybean leghemoglobin (77,96), but their mechanism of action is not understood. Other new proteins appear when nodules develop (76). These are called “nodulins” and are likely to be essential for symbiotic nitrogen fixation; however, their exact role is not known. Some of these might be enzymes, such as those for ammonium assimilation (67). When nodulins and their functions are better understood, a logical extension of current research will be to move cloned modulation genes into other plants. This may make it possible to extend nitrogen fixation to other plant species.

● **Two other free-living nitrogen-fixing bacteria,** ***Azosporillum*** and ***Azotobacter,*** also are **important agriculturally.**

Summary. -Individual nitrogen-fixing systems can be improved or extended by a knowledge of how they work and by techniques that permit the genes for nitrogen fixation to be altered and moved. One line of research will be the improvement of existing systems. Some new nitrogen-fixing systems have been proposed, as well. Proposals have been made, for example, to insert directly the genes for nitrogen fixation into the plant genome. Success of these as well as other systems in the end will be measured by the practicality of the new association, The problems of specificity, oxygen regulation, and effect on yield must be considered, and these will require broad-based knowledge of biochemistry, genetics, and physiology in a variety of nitrogen-fixing organisms (see table 37). There is a considerable amount of research being done in the area of nitrogen fixation, and genetically manipulated ***Rhizobiwn*** maybe field tested soon (85).

MICROBIALLY PRODUCED INSECTICIDES

Problems or drawbacks associated with chemical insecticides, including their increasing cost and environmental hazards, their lack of specificity, and the ease with which insect resistances to such insecticides are developed, have sparked renewed interest in microbially induced insect control to improve crop yield. Microbial insecticides, because of their narrow host ranges, can control specific pests while allowing natural predators and beneficial insects to survive. Furthermore, the few characterized microbial pesticides do not appear to harm humans or animals, and they are biodegradable.

There are three natural sources of microbial insecticides: bacterial, viral, and fungal. About 100 bacteria have been reported to synthesize toxins that are insecticidal. Very few of these bacteria have been studied extensively, but in one case (Bacillus ***thuringiemis kurstaki),*** the gene that controls the synthesis of a toxin has been cloned using rDNA technology (69). The cellular mechanism of the toxin's insecticidal activity is not yet well understood. Genes for bacterial toxins could be put into other bacteria that normally exist on the surface of plants (48).

Viruses also can be insecticidal by virtue of their ability to cause disease in various insects. Several families of viruses have been identified as potentially pathogenic to insects, but the family ***Bacu-Zoviridae*** has received the most attention. The U.S. Environmental Protection Agency (EPA) has registered, or is considering registering, several baculoviruses for the treatment of such diseases as cotton bollworm, Douglas fir tussock moth, gypsy moth, and alfalfa looper (81). One particular baculovirus ***[Autographa californica*** nuclear polyhedrosis virus (AcNPV)) has been genetically and molecularly well characterized, making the use of rDNA techniques with this virus feasible,

In contrast to bacterial and viral insecticides, fungal pathogens need not be ingested; they can disable or kill the insect by colonizing its surface. More than 500 fungal species can infect insects,

Table 37.—importance of Basic Research (Model Systems) on Nitrogen Fixation

Research area	Or9anisms used in research	Importance
Cloning nitrogenase genes.	*Klebsiella pneumonia*	Direct study of genes Introduction of nitrogen-fixing genes into other organisms
Physiology of nitrogen fixation	*Azotobacter* *Anbaena* *Klebsie/la*	Improving energy efficiency of nitrogen fixation in the cell Understanding role of ammonia in nitrogen fixation
Biochemistry of nitrogenase	*Clostridium* *Azotobacter* *Klebsiella* *Rhodospirillum*	Understanding oxygen sensitivity of n itrogenase Improving energy efficiency of nitrogenase enzyme
Cell and developmental biology of modulation	*Rhizobium*	Bacteriallplant recognition process Modulation process

SOURCE Office of Technology Assessment

and there are susceptible hosts in all the major orders of insects (72). The use of fungal insecticides will require a better understanding of their pathogenesis and ecological requirements. The large-scale production of these pathogens is difficult, and, in many cases, the technology is not developed. Also, their safety with regard to higher animals and humans has not been adequately studied (43,44)81).

DISEASE-SUPPRESSIVE AND GROWTH-REGULATING MICRO-ORGANISMS

Increasing plant yields may be achieved through better understanding of the many bacteria that protect plants from naturally occurring, deleterious conditions (80,92). Some of these bacteria act by producing compounds that bind iron. Others act by altering the pH or the salinity of the soil. Still others prevent frost damage to leaves. Furthermore, there are other bacteria that produce compounds that regulate plant growth. The mechanisms by which these processes occur is not well known. With better understanding of the genetics and biochemistry of some of these bacteria and their environment, it may be possible to program them genetically to produce compounds to change any number of soil and growth conditions.

For two reasons, it probably will not be economically feasible to incorporate the useful microorganisms directly into soil on a regular basis, because large amounts of the micro~organisms would be required and because the useful microorganisms might not be able to compete with the well-established microorganisms already present in the soil. Instead of being incorporated into soil, the useful microorganisms could be given a competitive advantage by applying them to the seeds or other plant parts prior to planting. Then they would already have established a niche allowing them an advantage over naturally occurring micro-organisms (92).

The first authorized deliberate release of genetically manipulated bacteria, planned for the fall of 1983, was to prevent frost damage. The genes coding for the compounds that initiate ice crystals were identified and deleted from a bacterial strain normally found on many crop plants. The researchers intended to spray these new bacteria on field crops early in the growing season, so that they became the established strain and crowded out the natural, harmful bacteria. * It is thought that this approach could prevent up to $5 billion worth of damage to crops throughout the world (80).

Conclusion

The first successes in DNA transformation of plants to give novel characteristics have been achieved. Continued research on vectors and plant tissue culture is needed to extend these successes from model systems to major crop plants. The identification of genes that would substantially improve a crop plant requires cooperation between traditional breeders and geneticists. Plant molecular biologists need the knowledge of the more traditional plant disciplines to produce agronomically useful plants more rapidly. Interdisciplinary basic research on plant biochemistry, development, and physiology will be required to help identify important genetic traits, to define biosynthetic pathways in plants, and consequently, to develop better plants. Many single gene traits in agronomic species have been used in past breeding programs; such genes also can be studied using new biotechnology, The novel technologies also can introduce genetic material from plants that normally do not interbreed and possibly provide simultaneous introduction of many specific traits into a single breeding line.

The next 5 years will produce major breakthroughs in DNA transformation in model systems and routine regeneration of plants from our major crop species. Problems such as changing the composition of the storage proteins of cereals and legumes are difficult, because multigene families limit the impact of single gene introductions. Within the next decade, however, genes conferring resistance to stress and disease are likely to be introduced and expressed in plants.

● **This** experiment was indefinitely postponed pending the outcome of a lawsuit filed against the U.S. Government raising the question of the necessity of filing an Environmental **Impact Statement prior to the deliberate release into the environment of a genetically manipulated microorganism.**

The production of better plants has obvious value in food production, but biotechnological developments in plants certainly will have other applications. Contributions to health care in the form of novel biological substances may result. Floriculture and the forestry industry will benefit from the development of new strains and acceleration of breeding programs. Plants could be used potentially as a source of industrial enzymes. The fiber industry is likely to see an increase in the production and quality of plant fibers, and an increased production of biomass (organic matter that grows by photosynthetic conversion of solar energy) should contribute to the generation of energy in the form of **ethanol (39). The production of energy from biomass is discussed further in *Chapter 9: Commodity Chemicals and Energy Production.***

Commercial aspects of biotechnology in plant agriculture

Although the generation of new plant varieties may be important to the farmer in increased yields or decreased costs of production or to the end processor **in better food products, the commercialization of plants developed using new biotechnology is in the hands of seed and live plant producers. The ability of the U.S. seed and plant producers to develop and market new plants will determine the competitive position of the United States in plant agriculture. In general, seed production** is not a business where international competition plays a role. Because the climates around the world vary so greatly, researchers would have to do field trials and grow seed in other countries. Thus, each locality generally does its own research and seed production.

Excellent research programs in the applications of biotechnology to plant agriculture exist in the United States, the United Kingdom, and Australia. Because of the climatic differences among these countries, the research is concentrated on different species.

The seed market is one of the largest markets to which biotechnology is directed. In the United States, $4.5 billion of seed are sold to farmers each year. Cuttings of vegetatively propagated plants account for $500 million of this market, and the largest segment of the market, $1.2 billion, is for corn seed. The world market for seeds is estimated at $30 billion. The United States exports $250 million of seeds per year, and U.S. **subsidiaries overseas contribute to world seed production (85).**

Another potentially lucrative market is the market for cut flowers. This market may be one of the easiest horticultural markets to enter with novel plants produced by biotechnology because it readily accepts and depends on novel phenotypes.

It is likely that genetically manipulated plants may increase the demand for commercial seeds. Drought-resistant plants could increase the acreage planted, and other plants might be planted at higher density, both resulting in an increase in the number of seeds sold.

A phenomenon not necessarily related to biotechnology is the long-established movement by U.S. farmers toward buying new seeds every year, rather than saving and planting seeds from crops produced the previous year. The evidence is gathering that seeds from companies give better results than a farmer's own seeds. Because the cost of seeds is only 3 to 7 percent of agricultural direct costs, it behooves the farmer to get the best seeds possible (85,100). The U.S. soybean industry, for example, has moved recently from buying approximately 20 percent of its seeds a few years ago to buying approximately 40 percent of its seeds today (85). This trend could amplify the demand for seeds produced by biotechnology.

The industrial production of agricultural chemicals now produced by micro-oraganisms or plants could be a substantial market. These pesticides, along with pest-resistant and nitrogen-fixing plants, could begin to capture the $10 billion domestic agricultural chemical market (78).

U.S. corporate investment in agricultural research has been high in the last few years. Many of the firms that have invested in plant biotechnology are chemical firms, especially firms that produce agricultural chemicals. The investment may be a response to a potential decrease in the agricultural chemical market due to the development of plants not needing chemicals (e.g., nitro-

gen-fixing plants, pest-resistant plants), the development of biological pesticides, or the development of plants with enhanced responses to chemicals. Another major industrial sector investing in plant biotechnology in the United States is the petroleum industry. The firms in this sector may see plants as the next source of energy, either in the form of biomass or photosynthesis itself. Pharmaceutical and food companies also are investing in plant agriculture. How the large chemical and petroleum corporations, the existing seed companies, and the NBFs will compete for market shares is yet to be seen.

The seed and vegetative cutting market is very large, and it appears that U.S. companies are oriented mainly toward domestic markets because of the transportation costs and the expense and inconvenience of field trials in other countries. Probably because of large domestic markets, many new entrepreneurial firms are directing their efforts toward plant agriculture. In fact, the number of NBFs in plant agriculture is third only to the number in pharmaceuticals and animal agriculture (see ***Chapter*** 4: ***Firms Commercializing Biotechnology).***

Priorities for future research

Animal agriculture

The prospects for the application of biotechnology in the areas of animal and plant agriculture are truly exciting. To encourage the introduction and progress of biotechnology in animal agriculture, however, several persistent problems must be overcome. These problems include the following:

- developing effective delivery systems for almost all products of new biotechnology to be used in animals;
- achieving consistent expression of polypeptides such as those used for subunit vaccines from rDNA systems;
- developing host/vector systems that yield products more closely resembling mammalian molecules (e.g., glycosylated proteins) and that secrete products for easier purification.
- demonstrating product stability under the climactic and handling conditions where these products (e.g., subunit vaccines) will be implemented; and
- achieving higher immune responses with **subunit vaccines, for example, by developing delivery systems that prolong exposure to the vaccine.**

More basic knowledge about biological processes in animals and about the cellular and molecular biology of pathogenic bacteria and animal parasites is required before many biotechnological applications are realistic. Advances in basic knowledge about metabolic pathways in beneficial bacteria may lead to useful growth-enhancing compounds. Finally, more basic knowledge concerning the actions of nascent products such as rDNA-produced GH is needed to discern effectiveness and safety.

Given the novelty of disciplines such as molecular genetics and cellular biology in animal science, there is some question as to whether sufficient communicative links are established yet between basic and applied scientists. The efforts of applied scientists usually are communicated to animal growers in the United States through the land grant universities' State Agricultural Experiment Stations and extension services, supported by USDA. A corollary to the productiveness of future research rests in encouraging the establishment of communication between basic and applied scientists to encourage biotechnological applications in animal agriculture.

*Plant agriculture **

Because interest in plant molecular biology is fairly recent, the most important research priority is an increased understanding of DNA structure

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"Research goals similar to those outlined in this section were published recently by the National Research Council (83) and the National Academy of Sciences (82).

and gene **expression in plants. * The knowledge generated from investigations of DNA sequences and their functions will be essential to the use of biotechnology in crop improvement, although the initial contributions of biotechnology will not be in crop improvement but in acquiring a better understanding of the basic biology of plants.**

It is unlikely that results from laboratory "model" species can be extrapolated to agriculturally important crop plants. Therefore, research is needed for improving and understanding the laboratory culture conditions for cells from these important plants. These plants must be able to be regenerated from single cells on a routine basis before many experiments using novel biological techniques can be performed. Much more work needs to be done before any plant cell vector can be used routinely. Additionally, a continued search for vectors for monocots is necessary if rDNA technology is to have an impact on some of the most important crop species.

It also is important to develop better selection methods. For instance, it is essential to be able to determine rapidly which cells carry specific genes and whether or not those genes are acting appropriately.

Both basic and applied research efforts in improved plant characteristics are quite active. The economic impact of finding disease or environmental resistances in the near term are potentially great enough that this research area is the primary thrust of many of the new plant genetics companies in the United States. Considerable effort continues in universities, as well, although overall funding for the university effort probably is much less than that represented by the current industrial effort. For many desirable traits, the actual protein product of the gene is not known. Cloning and genetic analysis of such genes would greatly increase the knowledge of what kinds of proteins are involved in disease and other resistances. Other improvements in specific plant characteristics may be made by modifying genes in major crop plants or by the introduction of novel traits from other plants. Both approaches warrant investigation.

Plants are known to produce a variety of secondary metabolizes that have either pharmaceutical or agricultural uses, yet little is known about the genetic regulation of their production or the development of culture systems for optimal production. Better understanding of these areas could lead to the production of new, improved, or less expensive drugs and compounds that attract or repel insects for controlling weeds and pests.

Goals for improved biological nitrogen fixation include extending nitrogen-fixing bacterial systems to a wider variety of plants, transferring the bacterial nitrogen-fixing genes to plants, and making existing nitrogen-fixing systems more efficient. Genetic studies will reveal how nitrogen-fixing genes are regulated, including how they respond to environmental levels of nitrogen and oxygen.

The extension of any of the nitrogen-fixing sysems depends partly on understanding more about survival and competition of nitrogen-fixing bacteria in field conditions. Temperature extremes, nutrient and pH status of soils, and presence of other micro-organisms are factors that influence colonization of host plants. Reliable, analytical descriptions of the field ecology and physiology of nitrogen-fixing organisms are needed.

Much basic biology of microbial insecticides is yet to be understood. In order to determine the appropriate strategy for their use, it is necessary to study the influence of such factors as sunlight, temperature, rain, and relative humidity on the microorganisms. Additionally, little is known about the mode and schedule of application and dose required for effective use of microa--ganisms in the field. Criteria established by EPA require an analysis of the pathogen's possible effect on human and animal health and the environment.

Even with the lack of biological knowledge currently, it is possible to apply the techniques of biotechnology to the field of microbial insecticides. Approaches include the development of more potent strains, an increase in their tolerance

● '1'11['S(' priorities only cover the techniques discussed in this report It should be noted, though, that genetic advances and applications are dependent on concurrent research in plant biochemistry and physiology

to environmental stresses, and an extension of their host ranges. The cloning of the *Bacillus* toxin gene, for example, opens up possibilities for the genetic manipulation of this gene to produce a more potent toxin and for the transfer of the gene to other microaganisms.

The virus AcNPV currently is well enough characterized that its use as a vector is now possible. Some ideas for genetic manipulation include the introduction of insect-specific toxins and broadening the host range of the virus. The use of fungal insecticides requires a better understanding of the physiology, genetics, and pathogenicity of the genes that code for these insecticides. This understanding should lead to the development of strains with increased virulence and greater ease of production in culture (81).

Plants are capable of producing insecticides, yet little is known about their biosynthesis or mode of action. Further research on this topic would allow for more specific, effective, and environmentally sound insecticides.

Because of the complexity of the photosynthetic system, more basic research is needed on the enzymatic processes of photosynthesis and their regulation and compartmentalization. Photosynthesis is used for the production of carbohydrates, and understanding how these compounds are partitioned throughout the plant may allow the ability to direct them into the harvestable parts of the plant.

Finally, knowledge concerning the ecological results of growing plants more densely or of growing plants on marginal land is scant. More research is needed on soil and water use and mineral cycling plants.

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