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**ABSTRACT**

To aid in the effort to define comprehensive long-range planning goals in bioregulation, the Agricultural Research Service (ARS) asked the Board of Agriculture of the National Research Council to undertake a study of the ARS research programs concerned with bioregulation. (For the purposes of this study bioregulation was interpreted broadly to be basic studies of key processes in the biosciences). This document presents specific program objectives for long-term research in the areas of: (1) genetic engineering; (2) food animals (with references to disease, growth and metabolism, and development and reproduction); (3) crops (citing needs relating to carbon and nitrogen input, growth and development, and physicochemical stress); and (4) plant diseases and insect pests (including plant-pathogen interactions, biological control, insect neurobiology, and pesticides). In addition, an attempt is made to define an optimal climate for basic research to occur. It is recommended that ARS implement a comprehensive program which would help create this climate for productive research. A list of ARS laboratory groups visited is appended. (TW)

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# New Directions for Biosciences Research in Agriculture

*High-Reward Opportunities*

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# New Directions for Biosciences Research in Agriculture

*High-Reward Opportunities*

Committee on Biosciences  
Research in Agriculture  
Board on Agriculture  
National Research Council

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NOTICE: The project that is the subject of this report was approved by the Governing Board of the National Research Council, whose members are drawn from the councils of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. The members of the committee responsible for the report were chosen for their special competences and with regard for appropriate balance.

This report has been reviewed by a group other than the authors according to procedures approved by a Report Review Committee consisting of members of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine.

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## Preface

In April 1982 the Agricultural Research Service (ARS) of the U.S. Department of Agriculture began a major ongoing review by sponsoring an internal symposium aimed at defining comprehensive, long-range planning goals in bioregulation. The agency also recently completed a program document that includes an accompanying six-year implementation plan focused on more immediate goals in research.<sup>1</sup>

As a part of this ongoing review and planning process, Terry B. Kinney, Jr., administrator of the ARS, requested that the Board on Agriculture of the National Research Council undertake a study of the ARS research programs concerned with bioregulation. Administrator Kinney asked that the board identify and recommend ARS programs in bioregulation that should be initiated or strengthened to ensure the highest dividends to agriculture. In the organization and execution of this request, bioregulation was interpreted broadly as basic studies of key processes in the biosciences.

The Board on Agriculture appointed a committee of 18 members with wide-ranging expertise to undertake this study. The Committee on Biosciences Research in Agriculture represents a breadth of knowledge across the disciplines of science and also represents a combination of experience in research, management, and administration in both academe and industry.

The committee was divided into three subcommittees to explore current and proposed ARS research efforts on mechanisms that regulate the biology of animals, plants, and insects and plant

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<sup>1</sup>U.S. Department of Agriculture, Agricultural Research Service. 1983. Agricultural Research Service Program Plan: 6-Year Implementation Plan, 1984-1990. Miscellaneous publication number 1429. Washington, D.C.



pathogens, respectively. Committee members interviewed a large number of research scientists and laboratory chiefs during 23 separate visits to 19 of the 147 ARS research centers throughout the United States and abroad (see Appendix). Some of these included ARS units that are affiliated with universities.

Although it was not possible for subcommittee members to meet with all ARS scientists in each laboratory group, open periods were arranged during many site visits so that any ARS scientist who wished to present ideas on priority research areas had an opportunity to do so. At university-associated laboratories, discussions included some of the university scientists who were conducting related research.

The committee members sought, through discussions with ARS scientists about both current and future programs, to obtain a clear view of the present capabilities of the ARS and to specify how these capabilities might be augmented to take advantage of the newer biotechnologies. They also recognized the importance of making recommendations about the conditions that combine to create an optimal climate for research, based on visits to ARS laboratories and on general experiences with changing climates in some of the outstanding laboratories practicing the new biology.

Committee members were pleased with the open and enthusiastic discussions that took place at all centers. The interest, cooperation, and contributions of ARS scientists were exemplary.

It is significant to note that the final conclusions—on both research opportunities and the optimal climate for basic research—of the Committee on Biosciences Research in Agriculture were prepared in response to the request from ARS, but they apply broadly to the agricultural research community. The conclusions are based on the thoughts and suggestions of many of the ARS scientists themselves, coupled with the experience and ideas of the committee members. Although other reports have addressed new opportunities in agriculture, especially in the plant sciences, this report provides a uniquely holistic view of agriculture, generated by an integrated committee of plant and animal scientists.

Ralph W. F. Hardy  
*Chairman*

## Acknowledgments

The committee wishes to express its appreciation to the ARS area and center directors, laboratory leaders, and scientists at the 19 locations visited for preparing background materials and research summaries for subcommittee members prior to their visits, and for assistance in organizing the visits. The committee acknowledges the staff of the Board on Agriculture—Selma P. Baron, Staff Officer; Philip Ross, Senior Staff Officer; and James E. Tavares, Project Officer—and their support and guidance during committee meetings and subcommittee site visits. The committee wishes to thank Aida Neel, Project Secretary, for her technical support during meetings and in the preparation of this report.

The committee members wish to express special gratitude to James E. Tavares and Carla Carlson, consultant and editor of this report, for drawing our ideas and conclusions into final form.

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## Executive Summary

In the committee's view of basic agricultural research as it is conducted within Agricultural Research Service (ARS) laboratories and within organizations throughout the country, three important features determine program planning direction. These are (1) the quickening pace of discovery, (2) the development of new molecular and cellular techniques that enhance current research practices, and (3) the necessity of interdisciplinary collaborations to determine and understand the basic processes of nature, particularly as they relate to efficient plant and animal productivity and health.

In realizing how these and other factors will influence the agricultural sciences in the United States for several decades, the ARS has seized the opportunity to reevaluate the structure and substance of its research programs. In the following summary of recommendations the National Research Council's Committee on Biosciences Research in Agriculture suggests ways to focus currently strong basic ARS research programs and identifies areas demanding new or expanded emphasis that will help the agency accomplish its goals.

This review of newer molecular genetic techniques and traditional research methods is presented as a selected list of high-reward opportunities for agricultural research. It is not intended to be a blueprint for the structure of research direction specific to the Agricultural Research Service. Rather, the basic research approaches and goals outlined in this report can apply to the agricultural research community at all levels, both within and outside the publicly supported system.

## Setting Priorities

The committee recommends that the Agricultural Research Service use this report to assist in the identification and selection of specific program objectives for long-term research. The committee acknowledges that it is neither practical nor possible for the ARS to achieve leadership status in all areas of research discussed in this report. ARS can achieve research leadership by selecting high-reward research opportunities that build upon current research strengths within ARS. In some instances the ARS should develop new initiatives such as the planned Plant Gene Expression Center. In this case the ARS is taking the opportunity to establish scientific leadership in a program that will not duplicate existing public and private research programs.

Selection of program objectives will also depend upon the availability of scientific staff, technical and financial resources, and the need to respond to issues such as food quality, public health, and economic factors. Selection must also be based on an assessment of the areas of high-quality research that are being emphasized at other public and private research institutions.

Additionally, program objectives based on newer molecular genetic techniques must compete scientifically for available ARS resources and should not be established at the expense of productive science based on conventional technologies. Program objectives must always be measured by the quality of the scientific investigation and its potential contribution.

The committee further recommends that the ARS establish a process for periodic outside review and evaluation of the scientific quality of long-term program objectives.

## Research in the Biosciences

### Genetic Engineering

All of the disciplines comprising the agricultural sciences are influenced by genetics. The collection of genes that determines the properties of an organism can differ qualitatively from organism to organism. These differences have been demonstrated by classical genetic analysis and have been used to breed desirable qualities into agricultural crops and food animals. The newer molecular techniques that are giving scientists the ability

to isolate, clone, and study genes provide a detailed and precise way of increasing the understanding of plant and animal genetics. The ARS should particularly focus molecular genetic research on important crop plants and food animals and on the maintenance and use of germ plasm collections. Further, the ARS should participate in the invention and development of additional molecular techniques.

#### **Food Animals**

**Disease** Increased research efforts, coupled with the use of newer techniques, will make safer, cheaper, and more effective vaccines, diagnostics, and therapeutic products available within a few years. Necessary research that must be conducted in food animals includes study of the molecular genetics of the immune response; characterization of antigens of pathogens; development of the scientific base for subunit vaccine production; and isolation, characterization, and activity of immune modulators.

**Growth and Metabolism** An understanding, generated from the use of newer techniques, of the molecular bases of key processes in food animals such as pregnancy, growth, lactation, and egg production will contribute greatly to improved metabolic efficiency and product quality. Studies are needed to identify, isolate, and characterize the endogenous chemical mediators of metabolism and their mechanisms of action at the organ, cellular, and intracellular levels. Further research should focus on the definition of relationships between feed stuffs, microbial fermentation, nutrient availability, and uptake. Based on the knowledge gathered from these investigations, scientists must develop a means to manipulate the fundamental control systems in food animals, specifically in tissues such as muscle, adipose, and bone.

**Development and Reproduction** The new biological methods offer special opportunities to understand animal reproduction, which in turn should result in enormous gains in productive efficiency. To improve the current understanding of reproduction and the modification of differentiation, research must emphasize in vitro manipulation of gametes and embryos, the addition of



genetic information to gametes and embryos, studies of the genome at the molecular level, and oogenesis and embryonic mortality. The ARS, specifically, should establish a food animal gene bank to assist the research community by coordinating and fostering the storage and maintenance of DNA libraries, gene transfer vectors, and probes.

### Crops

**Carbon and Nitrogen Input** Improvement of the genetic and chemical understanding of the fundamental processes of carbon and nitrogen fixation in plants will provide the bases for new approaches to increase the productivity of crop plants. It is of utmost importance that molecular genetic studies of nitrogen fixation and carbon fixation be continued. Studies must emphasize the genetic determinants that control the partitioning of photosynthate between the harvested and nonharvested part of the plant. Specifically, research should focus on the development of plants with a superior ability to utilize nutrients via an improved carbon dioxide-fixing enzyme or by the incorporation of an efficient C<sub>4</sub> system into C<sub>3</sub> plants. Nitrogen fixation must be studied in both free-living prokaryotes and symbiotic systems with the goal of improving the process. The ability to fix nitrogen might be incorporated directly into crop plants, or symbiotic relationships might be extended to nonleguminous crops.

**Growth and Development** Plant hormones and phytochrome affect almost all aspects of development, from seed germination to flowering. Increasing evidence points to these substances as major factors in gene expression. As the molecular understanding of gene expression in plants increases, so too will the opportunities for identifying the mechanisms of action that plant hormones and phytochrome use to regulate gene expression. Research should emphasize the role of the biosynthesis and degradation of plant hormones and phytochrome, and other regulatory substances in major developmental stages, such as flowering, germination, and senescence, that influence crop yield.

**Physicochemical Stress** Physicochemical stresses such as drought, cold, heat, salt, and toxic ions are the main factors limiting expansion of food, feed, and fiber pro-

duction. Further understanding of these factors is the basis for increased production potential. Research must emphasize the primary sites of damage to the plant caused by a specific stress factor, the mechanisms employed by stress-resistant plants to avoid and tolerate stress, and the genetic bases of these tolerance mechanisms. More specifically, studies should focus on the mechanisms of water and solute transport, especially into and within the roots; the role of excessive light as a destructive agent under stress conditions; and stress-related changes in membrane properties.

#### Plant Diseases and Insect Pests

**Plant-Pathogen Interactions** A molecular understanding of plant-pathogen interactions should lead to more effective, environmentally compatible, and less costly disease control technologies. The molecular bases, including the genetics, of factors that determine resistance or susceptibility in host-pathogen interactions must be defined. The basic steps in the development of disease symptoms caused by the invading pathogen must be elucidated. Researchers must attempt to transfer resistance traits to susceptible crop plants or seek ways to cause resistance genes to be expressed.

**Biological Control** The use of microbes currently is only a small aspect of control of competing biological systems. The impetus of the new biology presents opportunities to significantly increase microbial control of plant pathogens and insect pests and to detoxify pesticide residues. Studies must be designed to identify and explore microbial agents that can control plant diseases and insect pests and to improve their effectiveness by conventional and newer genetic techniques. Scientists must expand knowledge of the basic biology of nematodes to further identify ways to perturb their reproduction and development. They must increase the understanding of microorganisms that promote plant health. New research must also emphasize the selection or engineering of microbes to detoxify organic pesticide residues.

**Insect Neurobiology** The potential adverse effects of insecticides on the environment and on human and animal health, in addition to increasing resistance in pests, call for development of alternatives to current insect

pest control. The insect neural system has been identified as a fundamental site for manipulations that should provide new opportunities for control. A great need exists for establishment of the first multidisciplinary program in insect neurobiology. Research must focus on the molecular biological understanding of the synthesis, regulation, and activity of pheromones, neuropeptides, ecdysteroids, and juvenile hormones and of their interactions in insect growth, development, and reproduction.

**Pesticides** A clear understanding of the molecular basis of pesticide action will provide opportunities to develop the next generation of pesticides to decrease crop losses during production and storage. This could be achieved by means that supplement the traditional synthesis and screening methods. Using interdisciplinary techniques, scientists must identify the sites of action of pesticides, including those of metabolic activation and detoxification. Further research must be directed toward the isolation and characterization of new natural chemicals useful as pesticides.

#### Optimal Climate for Basic Research

A clear definition of major research areas and long-term goals is important to the quality of research within the ARS. Equally important, committee members believe, is the definition and provision of conditions that foster high-quality research. The following points summarize steps that the ARS should take to create the optimal climate for productive research.

**Periodic Outside Review** An outside advisory council of 5 to 10 leading scientists should be created to provide regular program review and to suggest new directions in research for the agency. Subcouncils should be formed to meet more specific needs.

**Leadership** Additional capable scientific leaders are needed as laboratory chiefs within the ARS. They should be selected primarily on a basis of scientific excellence and secondarily on a basis of management potential. The National Program Staff too must provide strong support and leadership for creative research within a flexible framework. Open exchanges must be encouraged between the National Program Staff and laboratory scientists. To

accomplish this the National Program Staff not only must encourage open and frequent communications with ARS scientists but also must be receptive to the new ideas and new research directions emerging from scientists in the laboratory.

**ARS Centers** The committee supports the agency's plan for the new Plant Gene Expression Center and its focus on basic research on plant molecular genetics. The committee recommends, because of duplication of scientific efforts at a number of the 147 ARS centers, that the number of sites be reduced, creating an effective critical mass of researchers at the fewer sites. The advisory council, through input from its subcouncils, could make specific recommendations on consolidation and regrouping of research programs and sites.

**Staff and Activities** The committee recommends that the ARS expand its relatively new postdoctoral program, with the goal being to establish a steady state of 750 nontenured staff members. Nontenured staff would include postdoctoral fellows and senior staff fellows positioned within the most productive basic research programs of the ARS. The influx of postdoctoral researchers will foster a vigorous exchange of ideas and facilitate further interdisciplinary activities that are essential to the effectiveness of research using new biology techniques. The committee also recommends that the ARS employ outside appraisals in the review of all candidates for tenure. Review for tenured positions should occur five years after initial hiring for Ph.D.-level basic research scientists rather than one year after employment as is current practice.

**Budget Flexibility** Allocations for salaries should not exceed 75 percent of the total budget of any ARS center. Where purchase of expensive materials is particularly critical to the maintenance of high-quality research, funds designated for salaries might be as low as 60 percent of the total budget. The ARS should designate approximately 10 percent of the total budget of centers as flexible funds to support meeting attendance, research-related travel, and new exploratory opportunities. The attendance at national and international meetings by ARS scientists is critical and should receive a higher priority. The ARS should also encourage its scientists to take sabbaticals to develop new skills.

Outside Relationships The ARS is encouraged to establish additional relationships with strong university groups. Such liaisons will have the effect of raising the numbers of scientists in some of the smaller ARS laboratories to the critical mass required for productive, quality research. The ARS must also begin to explore research relationships with industry. These may include seminars, laboratory visits, and cooperative research. The ARS should reevaluate its relationship with the general public and intensify consumer education about the importance of agriculture to the health of the nation's economy and its people.

# 1

## Introduction

The outcome of the best science is unpredictable. But scientific research at times yields a unifying idea or theory--a key that revolutionizes the understanding of a specific area of science and opens the way to new discoveries and practical applications. This has just happened in biology with molecular genetics.

The development of genetic theory, the growing understanding of the DNA molecule, and the expanding capabilities in cell and tissue culture present scientists with a fresh starting point for progress toward unpredictable but potentially great rewards.

Just as the hand lens and its progressive refinement to the electron microscope allowed the visualization of the invisible, the tools of molecular genetics and tissue culture now allow the isolation and manipulation of invisible hereditary determinants. With these tools biology is evolving beyond the realm of the descriptive.

What scientists have come to understand thus far about plants and animals is impressive. This basic knowledge has been swiftly carried forward by application. The result is an overall increase in U.S. agricultural productivity of 240 percent in the past 50 years.<sup>1</sup> This increase is characterized by dairy cows that have more than doubled milk production per cow since 1950 and by grain production that helps to feed the growing world population.

What scientists will now be able to accomplish through the use of molecular genetic techniques is awesome.

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<sup>1</sup>U.S. Department of Agriculture, Agricultural Research Service. 1983. Agricultural Research Service Program Plan: 6-Year Implementation Plan, 1984-1990. Miscellaneous publication number 1429. Washington, D.C.

Using these techniques of the new biology, scientists possess the ability to visualize the gene--to isolate, clone, and study the structure of a single gene and study its relationships to the processes of living things.

The molecular genetic and recombinant DNA techniques are opportunities to be seized. They are tools, not an end in themselves. They can be employed to discover additional basic information about genes and the protein products that trigger a response to disease, regulate growth and development, or govern communication between cells and between organs. More broadly, these techniques offer opportunities to explore basic questions in genetics, biochemistry, physiology, immunology, and neurobiology in innovative ways and from new perspectives.

This report points to the great potential of molecular genetic techniques and suggests how they might be coupled with other current methods to yield new insights into studies of food animals, crop plants, and plant pathogens and insect pests. It emphasizes the usefulness of these techniques--as tools--in studying important biological questions. To be slow in acknowledging and employing the power of these tools would be to delay the progress of U.S. agriculture.

In addition to discussions and recommendations on the combined techniques that will benefit studies on animals, crop plants, and plant pathogens and insect pests, the report presents an outline of those most important conditions that can collectively provide the appropriate environment for this research. These conditions include the availability of funds, quality researchers, suitable facilities, and equipment, and, particularly, the presence of an attitude that encourages and supports scientific research of the highest caliber.

At times, individuals and institutions must try to predict the direction of scientific research to meet the pressing needs of program planning, funding, and organization. There is some danger in prediction. The implementation of a rigid program structure can lead researchers toward attempts to fulfill an inaccurate prediction rather than encourage them to follow the path of the important unanswered question.

This report does not predict outcomes. It identifies areas of research that the committee believes hold the greatest promise for increased understanding of the biology of animals, plants, and pests and increased agricultural efficiency and productivity for the United States.

## 2

# Molecular Genetics and Genetic Engineering

Fundamental advances in biology during the past 12 years have brought scientists to an understanding of inheritance at the molecular level. Two technically straightforward and basic techniques--molecular cloning and DNA sequencing--are valuable and precise methods in themselves that can be used to learn about the structure and function of genes.

These two techniques demonstrate an overwhelming synergistic effect: Cloning has made possible the isolation of pure DNA segments, and sequencing of the nucleotide bases that comprise a DNA molecule has made possible the analysis and characterization of those isolated segments. Thus, scientists now can routinely dissect the set of genes possessed by a particular organism and define location, arrangement, and structure. From this point any number of creative manipulations can be employed to learn more about the transfer of desirable genes and the enhancement of traits, including those of food animals and crop plants.

Combined with conventional plant and animal breeding techniques and the knowledge provided through the collaborations of geneticists, biochemists, immunologists, molecular biologists, pathologists, and virologists, the two techniques create a solid foundation for basic research and for application in treatment and in the diagnosis of both inherited and pathogenic disease.

Endless numbers of basic questions await answers: What are the precise mechanisms of expression of a gene? What prompts a gene to switch on or off? How does location of a gene affect its expression? The DNA-based



technologies only now are being used in earnest to address such basic questions. These questions should become major preoccupations for the most talented researchers.

### Structure, Organization, and Expression of Genes

Estimates of the total number of genes--the genome--in the nucleus of each cell of a crop plant or food animal range from 10,000 to 100,000. It is indeed remarkable that methods can be devised to isolate one single gene from among the thousands in the genome and manipulate it in ways that result in the expression of the gene trait in a recipient organism. The techniques leading to such gene expression are isolation, cloning, and transfer.

#### Isolation

The first step in a genetically engineered manipulation is to locate a single gene from among the thousands comprising the genome. Currently, researchers most often work with one of the few genes that have been characterized through past studies, for searching out the location of a gene not yet studied is much like trying to find a citation in a book without the aid of an index. It is an arduous task that researchers have rendered somewhat easier by the creation of gene libraries for organisms.

To prepare a gene library the DNA of the organism is cut, using selected restriction enzymes that recognize a specific sequence of bases and then snip the strands between particular bases. A series of different restriction enzymes can be used to snip the DNA until it is reduced to lengths of approximately one to several genes. These smaller segments are sorted using a process called electrophoresis and then cloned to produce a quantity of the genetic material sufficient for further analysis. Each of these segments of DNA--the gene library--can then be searched, one at a time, to locate the desired gene. The tool used to pinpoint the gene is called a probe.

The ordered pairing of nucleotide bases in the double helix renders each DNA strand complementary to the other. The ability of separate strands to bind to their complementary strand, a process called hybridization,

provides a powerful probe for locating specific genes. A probe is a length of DNA or RNA, usually containing a radioactive tag, that has a sequence complementary to that of the desired gene. The radioactive tag makes the probe easily identifiable after it has paired with the nucleotide bases of the gene. Probes can be made when the sequence of a protein is known--the protein that is the end product of a particular gene. Working backward through the steps of gene expression, the researcher can determine the nucleotide base sequence of the gene and then synthesize the probe.

In addition, chromosomes or segments of chromosomes can now be identified by various molecular and cytogenetic techniques as being carriers of specific genes. Use of these methods reduces the size of the gene library that must be searched to locate a gene.

### Cloning

Following isolation the gene is cloned, or duplicated, and inserted into its new host cell. To date, the method most often used to accomplish both is insertion of the gene into a bacterial plasmid. A plasmid is a small circle of DNA that exists separately from an organism's main chromosomal complement. A plasmid carries its own DNA replication sequence and usually maintains itself in multiple copies within the cell.

To clone a gene, the ring-shaped plasmid is cleanly cut open using a restriction enzyme. The restriction enzyme is also used to prepare a length of DNA containing an isolated gene. When the cut plasmid and the isolated gene are mixed together in the presence of DNA ligase--an enzyme that rejoins cut ends of DNA molecules--the isolated gene fragment is incorporated into the plasmid ring. Now as the repaired plasmid replicates, the cloned gene is also replicated. In this manner copious amounts of the cloned gene may be produced within the bacterial host cell.

Cloned genes have four major uses: (1) as research tools to study the structure and function of the gene, (2) in the manufacture of the protein product coded for by the gene, (3) in the production of gene copies for the transfer of a specific trait into a new organism, and (4) as diagnostic test probes for the detection of specific viral diseases in medicine.

### Transfer

Plasmids are not the only vectors, or vehicles, used to transport a gene into a new organism. A virus possessing natural gene transfer capabilities or a transposable element (a DNA sequence that has the ability to move from place to place within the genome and affect the expression of neighboring genes) also can carry the genetically engineered gene into its host. In addition, vector systems can be based on other means of moving genes such as microinjection of DNA into the cell nucleus or direct uptake of DNA by cells from their culture medium.

### Expression

One of the key uncertainties in gene transfer is whether or not the foreign gene will be transcribed to RNA and the RNA translated into the protein product in its new environment. The goal of these manipulations, gene isolation, cloning, and transfer, is gene expression. To be successful, an appropriate level and timing of expression must be achieved during the lifetime of the recipient organism. That is, function of the genetic process governing the periods when the gene is off (when no protein is produced) and when it is on (when protein is produced) is critical.

Only moderate success has been achieved thus far in transferring cloned genes into test plants and animals. Progress is hampered by a lack of vectors that can effectively carry recombinant DNA into a new host and of the regulation of expression in the transferred foreign genes. In vitro analyses can yield much basic information on factors contributing to successful genetic manipulations; however, in vivo studies ultimately must be conducted in both plants and animals as well as in microorganisms.

### Opportunities in the Plant Sciences

The knowledge base supporting genetic engineering technology for the transfer and expression of foreign genes in crop species is limited. Relatively few important plant genes have been cloned and sequenced. In part this extends from a lack of knowledge of the

biochemical pathways in plants; few important gene products have been isolated and purified to the extent that they can be used in developing probes for isolating the gene.

#### Gene Isolation

There is a major need for increased understanding of the genetic basis of important plant traits. This knowledge will come only through a concerted effort by plant geneticists, cytogeneticists, biochemists, and developmental biologists to search the germ plasm of major crop species and their relatives for agriculturally important traits. These traits then must be defined, in both genetic and biochemical terms.

Traits controlled by one or more major genes amenable to genetic engineering include selectivity for herbicidal action, some cases of disease resistance, and synthesis and regulation of plant growth substances, such as in dwarfism. Other traits might include the key regulatory steps in metabolic pathways, such as assimilation of nutrients and partitioning of photosynthate (the combined products of photosynthesis), tolerance to toxic metals, and possibly tolerance to various physical environmental stresses. In several cases where plant and bacterial metabolic pathways are similar and where mutants are available or can more efficiently be induced in bacteria, genes from bacterial sources may well be used in the genetic engineering of plants. Fatty acid synthesis, aromatic amino acid synthesis, biological nitrogen fixation, and carbon fixation are traits currently under investigation in a number of laboratories.

Transposable elements, bits of mobile genetic information, were first recognized in maize and are now known to be present in many different organisms. Because these elements can move from one location in the genome to another, they may be very effective vectors for recombinant DNA. Transposable elements can cause phenotypic instability; they turn off or otherwise alter the expression of neighboring genes. This ability makes transposable elements unique tools for the isolation and characterization of genes.

Specific transposable elements may be able to function in species other than those in which they occur. There are certain structural similarities of transposable elements in organisms as divergent as the fruit fly

Drosophila and the flax plant Linum, for example. The discovery and characterization of transposable elements in leading crop species could be very important in advancing the technology of gene isolation, the development of vectors, and the control over suppression of undesirable genes. Because of their enormous potential for use in genetic engineering, the search for transposable elements in important crop plants and the study of their structure and function are extremely important.

Transposable elements can be used to isolate genes when other methods, such as screening in bacteria, will not work. The strategy is illustrated by recent success in cloning maize genes. First, the progenies of a plant that contains identifiable transposable elements are screened for the absence of a trait possessed by the original plant, such as resistance to a disease. The absence of the trait suggests that the transposable element has moved to a position adjacent to, or in the middle of, the gene responsible for that trait. The DNA of such an altered plant is then isolated and cut with restriction enzymes. The transposable element, which has a specific and unique nucleotide sequence, is used as a probe to locate DNA segments that contain the transposable element's DNA. These segments are then isolated, cloned, and sequenced. The DNA flanking the element is suspected of being a part of or perhaps the entire gene responsible for the trait in question.

Transposable elements have potential for use, in a similar fashion, in turning off undesirable genes. Such a naturally occurring case of gene dysfunction caused by the presence of DNA sequences in the middle of a gene has been described in soybeans.

#### Gene Transfer

In animal and bacterial systems the availability and early characterization of viruses and bacteriophages that naturally integrate into the genome of the host aided in the development of viral vectors that carry recombinant DNA into these host organisms. Most plant viruses are RNA viruses; the genetic information is carried by RNA rather than DNA. Only two groups of plant viruses contain DNA as their genetic material. No plant virus, to the best of current knowledge, is capable of being integrated into a host's chromosome.

Research is under way to develop a number of vector systems for use in transferring recombinant DNA into plants.

**Plasmids as Vectors** Two naturally occurring systems in plants do involve insertion of DNA sequences into chromosomes. The megaplasmids, Ti (tumor inducing) and Ri (root inducing), are carried into host plant cells in nature by the soil bacteria Agrobacterium tumefaciens and A. rhizogenes, respectively. They produce the diseases crown gall (Ti) and hairy root (Ri).

These megaplasmids contain a small region of DNA called T DNA (transfer DNA), which is transferred by an unknown mechanism into the chromosome of the host plant. After researchers understood that the disease caused by these bacteria was the result of insertion of plasmid T DNA into the plant chromosome, these plasmids were adapted for use in the first-generation plant genetic engineering experiments. More sophisticated use of vectors, based on the ability of T DNA to insert into chromosomes, will be possible once the molecular mechanism of the transfer is understood. While the diseases caused by these bacteria are found only in dicotyledons, the transfer mechanism also might be made to work in monocotyledons, including some economically important grain crops as well as in those dicots that are not susceptible to crown gall.

Little is known about the target site for insertion of T DNA. The limited evidence available suggests that there is not a specific insertion site--a potential disadvantage because of the importance of gene location for expression. This problem might be solved by modifying the T DNA or adding other sequences to the T DNA to make it specific for a single insertion site.

**Transposable Elements as Vectors** Transposable elements also have the ability to insert DNA into plant chromosomes. The expression of a gene adjacent to a transposable element on the chromosome is either stimulated or suppressed by the presence of the element. A transposable element also may carry its own functional genes that might encode an enzyme for transfer of the element itself. Further research is needed to assess the potential of transposable elements as vectors for plants. Important research goals within the next few years are to understand differences between active and

vestigial elements; element interaction and movement; circumstances governing the target site; and the meaning of the large, complex DNA sequences in the interior of some of these elements.

Viruses as Vectors As previously noted, plant viruses have been of marginal use thus far in plant genetic engineering. A better understanding of the genome structure of the few DNA-containing viruses and the many RNA plant viruses may lead to new and more promising possibilities. Such viruses might be developed as suitable vectors for in vitro assays that can quickly indicate the expression of a transferred alien gene. In addition, viruses might be used as cloning vectors to produce large amounts of a particular gene product. For example, as an economical alternative to the production of high-value biochemicals via cell cultures in fermenters, genetically engineered viruses might be developed to infect the crop in a farmer's field with the ability to increase the synthesis of necessary biochemicals prior to harvest. Viruses or viral sequences might be used to increase the efficiency of gene transfer. After entering the cell the recombinant DNA-containing viral sequence could replicate, increasing the probability that one or more copies of the gene would be integrated into the genome.

Attempts to insert DNA into the cauliflower mosaic virus, thought to have potential as a replicating vector, have had little success. The virus is apparently too small to accommodate most genes. Cauliflower mosaic virus commonly attacks members of the cabbage family and causes banding of veins in the leaves of the plant. Very recently a small bacterial gene encoding the enzyme, dihydrofolate reductase (dhfr) was inserted into cauliflower mosaic virus. Turnip plants became systemically infected, following inoculation with the recombinant virus, and acquired resistance to methotrexate. This resistance is a trait conferred by the activity of the dhfr enzyme.

#### Other Vectors: Microinjection and Direct DNA Uptake

Other vector approaches in plants are currently under investigation. Chief among these are microinjection and direct DNA uptake.

Microinjection, as a means of introducing DNA into the cell nucleus, has been successful in animal embryo systems. A few picoliters of fluid containing recombinant

DNA can be injected into a plant cell, and even into the nucleus, with fine glass pipettes. The cells then can be cultured. To date, no confirmed transformation of a plant species by this approach has been reported, but results are expected soon.

Microinjection technology will be important in the transfer of chromosomes in advanced cytogenetic manipulations and possibly also for the transfer of genes into organelles. Investigations in these areas offer opportunities for research collaboration among molecular biologists, cell biologists, and biophysicists.

In direct DNA transfer, DNA is taken up by cells from their culture medium and is integrated, by unknown mechanisms, into the chromosome. Such methods work in bacteria and animals. Similar approaches have so far proved less successful in plants, but the situation may be changing. It has long been known that plant viral RNAs and DNAs can be taken up in a biologically active form. The same has been shown for T DNA, but at a lower efficiency. It is possible, but not yet widely accepted, that lipid vesicles or analogous vesicular structures made from plant membranes might increase the efficiency of delivery of DNA as they fuse with the recipient cell membrane.

These latter methods are attractive and important areas for further investigation. They should be applicable to all plants and they avoid incorporation of the accompanying DNA of a potentially pathogenic vector.

#### Cell Culture and Plant Regeneration

As important and exciting as the recent advances have been in developing vectors for use in plant gene transfer, major challenges remain. A useful gene transfer system requires the ability to manipulate the cells of a species so that alien DNA can be inserted in a way that does not kill the cell. In addition, the cell must develop into a viable, functioning plant that has not been altered in undesirable ways.

Plant organ and tissue culture is a well-established technology that originated in the early part of the twentieth century. In certain ornamental and woody species, use of tissue culture for propagating new plants is a small but important agricultural industry. Progress in manipulating cultures of major food crops, particularly the cereals and legumes, however, has been much slower. Chapter 4 of this report addresses the



rather thin scientific basis supporting the current knowledge of organogenesis and plant developmental biology. It is important to note here, however, that the current inability to successfully regenerate, at will and at high frequency, whole plants from individual cells of major crop species severely limits use of even current gene transfer technology. Much of the sophisticated cell culture and related technologies required to undertake state-of-the-art gene transfer research in major crop plants is largely in the hands of a small number of industrial laboratories. The deficiencies in fundamental knowledge of plant development will become even more serious in the future unless a major research commitment is made by the public sector.

An alternative to the use of single somatic cells for genetic transformation is the insertion of genes into pollen nuclei, ovules, or recently fertilized embryos. By using gametes or developing embryos instead of somatic cells, both the potential for unwanted mutations from prolonged in vitro culture and the problem of regenerating a whole plant containing the new genes would be avoided. Nevertheless, the development of a firm scientific and experimental basis in the physiology, topology, biochemistry, and genetics of plant morphogenesis, including normal and somatic embryogenesis, will make an important contribution to several areas of agricultural biology, not least of which is the area of gene transfer.

### Gene Expression

The comparison of gene structures has yielded some insights into the factors governing expression of plant genes. What is known about expression, however, is greatly exceeded by what remains unknown. The recent success of gene transfer experiments using T DNA as a vector will dramatically quicken the pace of research on factors affecting gene expression in different plants. Further experiments will enable scientists to dissect the DNA regulatory sequences that flank the coding region of a gene--that segment providing the on and off signals for the transcription of DNA. After making changes in the nucleotide base sequence of these regulating, flanking regions, scientists can study the consequences by measuring the expression of the gene when it is put into the chromosomes of different plants. This type of study,

which ideally would include experiments with the same gene and flanking sequences in differing plant species, requires a major commitment of time and expertise.

**Effect of Location on Gene Expression** Experimental evidence indicates that factors involved in directing gene expression reside in the immediate flanking sequences. Equally important signals, however, may be present in the coding region of the gene itself and also in sequences some distance from the gene, or even on different chromosomes. The transformation technology currently available is insufficiently precise for use in targeting an insertion to a specific location in the chromosome. Thus, the possibility that location may be an important factor in governing gene expression must be addressed by repeated experiments in which several different insertions of the same gene are made at various locations. The same gene inserted in a single copy at one location may be regulated quite differently than when inserted in multiple copies at the same locus or in multiple copies at different loci.

**Regulatory Sequences** The regulatory signals controlling gene expression in bacteria differ from those in plants. Results of limited work to date indicate that sequences regulating gene expression in animals and animal viruses do not function in plants. Whether such sequences in one plant genus or family will always work in others is not yet known. Regulatory sequences in T DNA do function throughout a wide range of plant species that span many families. To a more limited extent, the same is true for cauliflower mosaic virus; regulatory sequences from this virus, when used in a T DNA-based transformation system, have been demonstrated to function as a regulatory signal in genera that are not considered to be hosts for the virus. The regulatory sequence flanking the nuclear gene that encodes a small subunit of the photosynthetic enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase in peas also functions in the petunia. In other cases, however, regulatory sequences fail to correctly control gene expression in unrelated species. Failure is tentatively attributed to an as yet poorly understood species specificity of the regulatory sequences.

Most genes are turned on and off at specific times in development or under special conditions. In various laboratories the expression of such genes is now

beginning to be studied. Regulatory sequences flanking important genes that are known to be triggered by light, heat, or growth hormones, for example, can be isolated and fused to a reporter gene. The reporter gene, usually a microbial gene carrying the trait for resistance to an antibiotic, provides a tag that can be used for screening and locating cells or plants that have incorporated the regulated gene sequence. The regulation of the transferred gene can then be tested by looking for its expression in the appropriate tissue or by triggering its expression using the appropriate environmental stimulus. This work, however, is in its most preliminary stages.

**Transient Expression Assays** Gene expression research would be greatly aided by a system in which genes could be expressed and assayed quickly within plant cells. The current system using the Ti plasmid requires weeks to months to obtain results from a gene transfer experiment. A so-called transient expression assay system might be developed by using modified plant viruses as promoter vectors for individual plant cells. The ability of an inserted gene to be transcribed and translated could be quickly assayed in a single cell by using sensitive hybridization and antibody probes to look for the messenger RNA (mRNA) and protein product of the inserted gene. The mRNA carries the code for a particular protein from the DNA in the nucleus to the cytoplasm. There it acts as a template for the formation of that protein.

Such an assay system would significantly advance the science of plant genetic engineering, because even small adjustments to sections of the transferred gene could be tested within a matter of days to find the nucleotide sequence that will be expressed in the host plant. The stability and function of foreign gene products, including enzymes and other proteins, could be tested quickly using such a system.

**Multiple Gene Traits** For many years plant breeders and cytogeneticists have obtained novel gene combinations by crossing certain distantly related species of the same or a closely related genus. Often such wide crosses involve an increase in the ploidy level to include duplication of the chromosomes from both parents. An example from nature is wheat. It has been shown that wheat is a hexaploid resulting from crosses among three genera:

Agropyron, Aegilops, and Triticum. Much has been learned using these breeding and cytogenetic methods.

The development of microinjection and other such vector technologies, improvement in fluorescence-activated sorting technology to refine methods for isolating chromosomes, and the construction of artificial chromosomes, so far only achieved in yeast, may provide future means for the transfer and expression of agriculturally significant complex genetic traits to yield new genotypes. As experimental tools, these methods will lead to advances in our understanding of coordinated gene regulation; as practical tools, they will lead to more rapid product development. These methods also will make possible the genetic engineering of plants for complex quantitative traits such as yield, disease resistance, and production of important secondary products such as flavors, fragrances, and pharmaceuticals.

#### Research Status

Basic research of a multidisciplinary nature is required to isolate, analyse, transfer, and express plant genes using modern biotechnology methods. The research requires expensive materials and some expensive equipment. Optimal use of resources and the multidisciplinary nature of the work dictate a concentration of effort and resources rather than a diffuse, decentralized organization.

The ARS must take a strong lead in both basic and applied research in plant genetics to sustain agricultural growth and prosperity in the United States. The agency must be particularly committed to focused research on important crop plants, the maintenance and use of germ plasm collections, and the high-risk, multidisciplinary research that is essential in bringing newer biotechnologies into practice.

To improve the available technology and the efficiency of gene isolation and molecular cloning in plants, special attention should be directed toward the following:

- Characterization of the biochemical basis and genetic traits involved in important plant processes such as photosynthesis, carbohydrate partitioning, yield, heterosis, stress tolerance, and morphogenesis;
- Molecular characterization of mobile genetic elements, such as transposable elements, plant viruses,

and plasmids, and properties such as host range, target sites for insertion into the chromosome, and the basis for the genetic dialogue between genes of the nucleus and organelles;

- Understanding of basic chromosomal structure and function underlying conventional cytogenetic manipulations, such as the creation of allopolyploids with wide crosses, and development of principles to guide the use of novel methods, such as microinjection and cell fusion, to manipulate chromosomes or parts of chromosomes;

- Understanding of the principal molecular factors and DNA sequences underlying the regulation of gene expression, such as mechanisms associated with chromosomal structure, sequences flanking coding regions, signals within coding regions, and functions of introns;

- Development of vector systems for transient expression assays.

Currently some of the strongest basic programs in plant molecular genetics are located within the research laboratories of private companies. This is particularly true for research on gene transfer systems for plants. Research programs on plant gene isolation and structure at universities and other publicly supported research laboratories usually consist of only one or two principal investigators. Public support of basic plant genetic research needs increased attention. The creation of the Plant Gene Expression Center at Albany, California, is a first step in this direction.

#### Aspects of Molecular Genetics of Food Animals

The knowledge base supporting genetic engineering technology for animals is extensive. Much of the biochemical and molecular genetic understanding of mammalian systems has been achieved through research on human cell culture lines and the laboratory mouse. Discoveries made using these laboratory systems are generally applicable to food animals. The application of these new techniques, however, remains limited; the nucleotide sequences of most of the genes coding for valuable agricultural traits and regulation of the expression of such genes remain unknown or are poorly understood.

Specific opportunities to apply molecular genetic techniques to the study of metabolic regulation,

reproduction, and functions of the immune system and to the development of vaccines, and diagnostic and therapeutic agents for food animals are discussed in Chapter 3. In addition, basic approaches to the study of gene isolation, transfer, and expression are covered in the previous section on plants.

This discussion outlines the principal methods used to introduce recombinant genes into the genome of food animals. It presents the potential advantages offered by analysis of the nucleotide sequence of genes and the mechanisms regulating their expression in food animals for the improvement of agricultural efficiency.

### Gene Transfer

Unlike plants, which can be propagated asexually, a whole animal cannot be regenerated from a single somatic cell. To introduce cloned genes into all cells of an animal, they must be inserted into the undifferentiated embryo. An alternative approach is the introduction of recombinant genes into the developing embryo or into somatic tissues, using retroviruses or transposons as vectors. With introduction into somatic tissues, however, germ cells will usually not be genetically altered, and recombinant genes will not be passed on to the offspring.

Microinjection into the Germ Line The stable integration of foreign genes into the mouse genome has been achieved by microinjecting cloned genes into the one-cell mouse embryo. The period following fertilization of the egg but prior to mixing of the genetic information of the sperm and egg appears to be an opportune time to incorporate foreign genes into the genome. Successful incorporation of the recombinant DNA at this one-cell stage establishes the foreign gene throughout all cells in the resulting animal, including cells of the germ line that give rise to future generations.

Mouse populations have been produced that contain recombinant oncogenes or genes coding for thymidine kinase, rabbit beta-globin, human leukocyte interferon, chicken transferrin, or rat growth hormone. These genes have been integrated into the mouse genome, and protein products resulting from the expression of these genes have been detected. The regulatory sequence used was a

metallothionein promoter sequence fused to the rat growth hormone gene. As a result the regulation of its expression was not the same as in normal mice. The concentrations of growth hormone in some of the transgenic mice were greatly elevated, and as a result the animals grew substantially larger than normal mice.

Growth hormone supplied exogenously to mice and some food animals has a dramatic effect in increasing growth rate. In addition, feed efficiency and body composition, in terms of reduced deposition of fat, often are substantially improved. The extent of these effects appears to depend upon the stage of development of the animal. Younger animals do not respond to growth hormone treatment as markedly as do mature animals. And the effect of growth hormone on increased milk production in cows, for example, is most pronounced in low-producing dairy cattle. The results are encouraging and portend important future applications for the cattle, poultry, sheep, and swine industries.

Microinjection techniques that were developed to insert cloned genes into mice embryos should be applicable to food animals. Specific problems in manipulating the one-cell embryo in different species must be resolved. With poultry this may not be possible, because it will be extremely difficult to obtain and manipulate viable one-cell embryos. It may be possible, however, to insert foreign genes via the spermatozoa, which can be used in artificial insemination.

Retroviral-based Vectors The genome of a retrovirus consists of single-stranded RNA that, following inoculation, serves as a template for reverse transcription and the production of a double-stranded DNA molecule that integrates into the chromosome of the infected cell. Integrated DNA copies of RNA retroviruses are called proviruses. Proviruses are transcribed and replicated along with the host's genes.

The provirus contains special sequences at both ends of its DNA that permit it to be integrated into the cell genome in a manner similar to other movable genetic elements, such as transposons. It is theorized that retroviruses are, in fact, movable genetic elements that possess genes for coat proteins, and that a virus particle is created by enveloping the RNA transcript within the coat protein. The converse is also possible; movable genetic elements or transposons might have arisen from

retroviruses that lost the ability to form a virus particle.

Foreign genes can be inserted into the provirus DNA. Such recombinant provirus DNAs can be cloned and used as vehicles for inserting the foreign gene into a host animal cell. The advantage of proviruses as gene transfer vectors is the efficient, transposon-like mechanism by which they can be integrated into the chromosomal DNA of host cells.

Other Vectors In addition to retroviral vectors, non-lytic DNA viruses, such as bovine papilloma virus (BPV), are being experimentally tested as gene transfer vectors. BPV does not integrate into the host cell chromosome; it exists instead as an episome, a stable extra-chromosomal unit of DNA in the host cell nucleus. A transformed cell may contain from 20 to 100 copies of the BPV episome. It appears that some of the genes necessary for the oncogenic transformation properties of BPV are not needed for its autonomous replication in the host cell. The BPV vector appears to be an excellent candidate for rapid assays for gene expression, because DNA from a mammalian species can be spliced into the BPV and tested for expression in cultured cells of that same species. The multiple copies of the BPV episome in each cell may amplify the expression of any intact genes included in the spliced DNA.

Other methods for inserting recombinant genes have not been successful in one-cell embryos, probably because the uptake of recombinant DNA is less efficient than micro-injection and adequate testing would require enormous quantities of these embryos. These methods include the uptake of calcium phosphate-DNA precipitates; electroporation, or uptake through the cell membrane stimulated by electrical charges; and uptake by fusion with vector-containing liposomes.

#### Gene Identification and Cross Cloning

A relatively low reproductive rate coupled with the enormous expenses involved in maintaining large populations of food animals makes it difficult to carry out the extensive breeding experiments needed for classical genetic analysis and chromosome mapping. However, mapping at the DNA level is now a reality and



can be applied to food animals. One form of mapping that could be easily applied to food animals is analysis of the genome based upon restriction enzyme sites. Another is the analysis of the nucleotide sequence of genes. Gene libraries can be obtained easily for both approaches. In addition, the discovery of restriction enzyme polymorphisms would provide exceedingly useful markers for genetic analysis in animal breeding studies.

Additional information for identifying and isolating specific genes might be compiled through cross cloning, which makes use of a DNA gene probe from one species to hunt for a comparable gene in an organism belonging to another species or genus. A comparable gene should have some homology in its nucleotide sequence and therefore should hybridize with the DNA gene probe. For example, many of the identified genes available in the gene libraries of cultured human cells or the laboratory mouse could be employed as DNA probes to search for the same gene in food animals. There are many enzymes and gene products that are common to all mammals. This technique has been used extensively and successfully to locate and identify genes such as oncogenes and genes encoding globin, cytochrome, myosin, actin, tubulin, growth hormone, and interferons in a variety of organisms.

#### Gene Expression

The successful transfer of a functioning growth hormone gene into the mouse is significant in two important respects. First, it demonstrated that this gene could be cloned, microinjected into a one-cell embryo, and expressed as part of the genome of the resulting transgenic mouse. But it also emphasized the significance of types of gene regulation, because the mice grew substantially larger than a normal mouse. The DNA sequence encoding the gene product and the promoter DNA sequences encoding the regulation of the expression of the gene are both equally critical components of a recombinant gene.

The second important aspect was the effect of the inserted gene on growth. A complex biological process such as growth obviously involves the expression of many --perhaps hundreds--of genes, yet growth in this case was regulated by a single gene. The ability to regulate the endogenous synthesis of this key substance offers a means to control a complex process such as growth. There are

most likely many other single genes that code for the synthesis of the critical modulator controlling other complex, multigenic traits.

The growing body of evidence on gene regulation in eukaryotes suggests that genes can be regulated at many different levels. To add to this complexity, different genes may be regulated in different ways. For example, significant progress has been made in understanding the regulation of the globin genes in humans and other animal species. It is now known that modification of the DNA may determine the switch from one hemoglobin type in the fetus to another in the adult. Methylation of the DNA seems to be an important aspect of this regulatory process.

The regulation of gene expression in eukaryotes does not appear to be based on the operon system, which is the major regulatory system in prokaryotes. One problem is that the genes affecting a particular trait in eukaryotes are often not clustered according to their sequence of expression as in prokaryotes. Furthermore, eukaryotic genes often are regulated on a long-term, irreversible basis typical of cellular differentiation and development. It is apparent, therefore, that notable strides in understanding development will go hand in hand with advances in knowledge and the ability to manipulate gene regulation in food animals.

#### Research Status

Studies of the fine structure of genes and the mechanisms regulating the expression of economically valuable traits in food animals are now possible. Many of the gene transfer systems and methods for molecular genetic analyses that evolved from studies on laboratory mice and human cell culture should be applicable to similar studies on food animals. The ARS has a well-established research effort at Beltsville, Maryland, on gene transfer in food animals. This and related areas of molecular genetic research should be expanded during the next several years, with particular emphasis on the following:

- Characterization of the physiological basis and genetic traits involved in important animal processes such as disease resistance, the immune response, metabolic regulation of nutrient utilization, developmental biology, and other aspects of production efficiency.

- Development of methods to manipulate viable gametes and embryos of food animal species, and development of suitable gene transfer vehicles and methods for genetic transformation of food animals.

- Understanding of gene promoter sequences in food animal species and the factors and conditions that control their function. This will require the development of rapid gene expression assay systems for each species.

- Establishment and analysis of gene libraries for food animal genotypes. Mapping of restriction enzyme fragments, identification of DNA polymorphisms as markers, and sequencing of nucleotides of identified genes will be valuable resources for both animal breeding studies and molecular genetic research.

#### Potential Impact on U.S. Agriculture

Modern genetic technology, including recombinant DNA and the ability to isolate, transfer, and express foreign genes in crop plants and food animals, will likely have an impact on agriculture comparable to that of the discovery of the laws of inheritance in the late 1800s. Improved species with new capabilities might be developed. Equally important will be the efficiency with which new traits can be incorporated into superior, adapted crops and food animals, and the ability to produce novel combinations of traits that are difficult or impossible to create using conventional breeding methods.

This technology will greatly improve current understanding of the biochemistry and genetics of animal and plant growth, development, and reproduction. But the transfer of this knowledge to agricultural sciences is as difficult to foresee as was the development of sophisticated statistical models for modern plant and animal breeding from the basic gene theory of inheritance. While it is true that use can be made of a system before it is fully understood, experience shows that a mechanistic understanding can unveil unexpected opportunities to take full advantage of a technology. A detailed understanding can also mitigate potential negative effects of a technology. A fuller understanding in the 1940s of the potency of chemical mutagens, for example, might have reduced the improper use and disposal of earlier synthetic chemicals.

In the short term the new biological technologies will have a variety of important implications for agriculture. Interest in preserving germ plasm and in compre-

hensive screening for useful traits is becoming more widespread, due in part to the influence of genetic engineering. Increasing interest is also being generated in other areas of basic plant and animal sciences, including biochemistry, physiology, pathology, and development, where genetic engineering tools serve as key adjuncts to more traditional research methods.

### 3

## Animal Science

Opportunities for research advances that will improve the productivity of the livestock industries are greater today than at any time in history. The powerful new tools of biotechnology can be applied directly to the development of knowledge about food animals and the production of biologicals to enhance livestock productivity. In addition, many of the genetic and reproductive manipulations that are not possible in humans, either ethically or practically, can be accomplished in farm animals. Spectacular advances in disease and parasite control and striking increases in the efficiency of converting feed-stuffs to meat, milk, and eggs can be expected within one to two decades if animal science research is properly focused and supported.

Scientific livestock breeding already has increased production dramatically since the time herds were driven across the plains to market. As recently as 1950, 22 million dairy cows in the United States were producing more than 2,400 kilograms per cow of milk annually. Now, only one-half the number of cows are producing the same total amount of milk while consuming one-third less total feed. The objective is not to produce more milk, but rather to produce more efficiently, with a reduction in feed, feedlot pollution, and animal maintenance costs.

The turkey industry has been particularly successful in using quantitative genetic principles to produce big-breasted turkeys efficiently. Now the quality of these birds is controlled through the use of artificial insemination. In fact, 100 percent of the commercial turkey flocks in this country is replaced each year using artificial insemination. Other industries are using similar, more traditional techniques to improve production in cattle, hogs, and sheep while continuing to explore the

basic science of genetics to further understand and enhance genetic improvement.

The new biotechnology methods, as tools that enhance conventional breeding methods, make possible for the first time realistic consideration of such ideas as the genetic engineering of an animal, one possessing more desirable production traits. Segments of DNA coding for desirable genes can be isolated in the laboratory, inserted into suitable DNA-carrying vectors, and transferred into host animals or into a bacterial cloning system. Extra copies of the gene coding for growth hormone have already been inserted into mouse embryos, yielding offspring twice the normal size. Bovine growth hormone is now being produced in the laboratory by a bacterial cloning system that provides sufficient quantities of this scarce biological for experiments on growth and milk production in cattle. Such experiments have major implications for improving productivity in farm animals.

The exploitation of hybridoma technology--fusing a continuously dividing cell with one that produces antibody--in the production of monoclonal antibodies has substantially enhanced the identification and isolation of genes and gene products as well as the production of highly specific antibody preparations for diagnostic and therapeutic uses. Scientists must be provided with funds and facilities to take immediate advantage of these and similar developments to explore gene structure, function, and regulation, and the basic physiology of livestock species. The following discussions specify the kind of research that the committee believes will result in improved animal productivity.

#### Molecular Basis of Disease

Each year the productivity of livestock and poultry in the United States is reduced by at least 20 percent because of diseases. This represents an estimated annual economic loss of \$14 billion. Until recently the research approaches available to address disease losses in food animals have been limited. The availability of new technologies such as recombinant DNA and monoclonal antibodies now affords an exceptional opportunity to understand and control disease.

The ability to isolate and clone genes that play a role in immunity is an enormous step toward eliminating

certain diseases. Major advances relate to the growing body of knowledge about the genes regulating the immune response, the genes controlling the antigen components of diseases and parasites, and the ability to create hybridomas with immune cells that can yield highly specific monoclonal antibodies.

The payoff from intensified efforts in animal disease control will probably come much more quickly than results from research in either metabolic regulation or reproduction because of the solid foundations already being laid in this area for both man and animals. For example, studies at the ARS Plum Island Animal Disease Center in New York, on the molecular biology of foot and mouth disease, in addition to dissecting the physical chemistry and biochemistry of the virus, have led to trials of a promising vaccine based on the cloned surface protein of the virus.

By the very nature of infection and disease, the benefits of vaccines are immediate, while applications in genetic improvement are slowed by the necessity of analyzing results in subsequent generations. More importantly, knowledge gained from studies of a particular viral or microbial disease and development of a vaccine often can be readily extended to many other diseases.

#### Genes Regulating the Immune Response

The strides that have been made in molecular genetics have been matched by those made in immunology. Now it is possible to combine the principles and techniques of molecular genetics and immunology to address one of the fundamental questions in biology--the nature of the recognition and response mechanisms in immunity. The application of this basic knowledge can be immediately translated into means for protecting economically important animals from costly diseases.

Current intensive investigations in both laboratory animals and humans are now providing an initial view of the structure and function of three classes of genes that control the expression of the immune response. These are the genes coding for the major histocompatibility complex (MHC), for mediator proteins called lymphokines, and for antibodies. Very little research to date, however, has been conducted on the immune response in food animals.

There are two major white blood cell types, B and T lymphocytes, involved in the immune reaction. Viruses, bacteria, parasites, or other foreign substances contain immunologically active macromolecules called antigens. When an infectious agent enters the body, its antigens stimulate the immune system to produce specialized proteins, or antibodies, that can recognize and bind to the antigens. Each unique antigen triggers both B cells and T cells: B cells produce antibody specific to that antigen, and T cells produce an antibodylike T cell receptor, also specific to the antigen. B cells release the antibody into the blood, creating extracellular immunity, and T cells carry the T cell receptors on their cell surfaces, providing cellular immunity.

Exposure to additional antigen stimulates the B and T cells previously dedicated to that antigen to divide and produce their respective antigen-specific products. A vaccine is simply an antigen or set of antigens unique to a disease-causing organism that stimulates a specific immune response against the disease agent.

The interaction of the T cell receptor with antigen is unique in that recognition involves proteins of the MHC that also play an important role in helping to distinguish between foreign substances, which should be destroyed, and self proteins, which should not be destroyed. The host does not normally develop antibodies or T cells directed against its own proteins, although this does occur in certain autoimmune diseases.

**Major Histocompatibility Complex** A family of genes located on a single chromosome codes for the MHC. In addition to recognition in the immune response, these genes are associated with the inheritance of diseases that appear to be malfunctions in the ability to distinguish between self and nonself molecules. Rheumatoid arthritis, multiple sclerosis, and juvenile-onset diabetes are examples of MHC-related diseases in humans. In animals, Marek's disease, a blood cancer in chickens, and scrapie, a disease of the central nervous system in sheep, appear to have strong relationships to the MHC.

Certain strains of chickens with Marek's disease demonstrate a greater resistance to the development of lymphomas, or tumors, than do others. Immunogeneticists have noted that these strains have a given MHC, or set of closely linked genes. Similarly, recent studies on scrapie indicate that different breeds of sheep exhibit



different degrees of expression of the disease. The severity of the disease appears to be associated with the transmission of certain MHC gene components.

Efforts should be intensified to elucidate fully the genetic makeup of the MHC in food animals and to define the role of gene products in regulating host immunity and susceptibility to disease. Direct application of the findings from such basic research will allow for the development of improved breeding programs. Specifically, breeders will be able to select for greater resistance to various diseases, thus upgrading the herd or flock and reducing the high direct or indirect costs of disease treatment and loss.

Lymphocyte Hormones As part of the immune response, lymphocytes can be directed to secrete soluble peptides that stimulate or suppress antibody production, division, and similar activities in other cells. These mediators, or hormones, are called lymphokines. In recent studies of such substances, interleukin-2 was shown to promote lymphocyte replication, which has greatly facilitated the in vitro growth and cloning of T cells. Another lymphokine, interferon, already has found clinical application in the treatment of some cancers and viral diseases in humans, and is undergoing field trials as a preventive measure for bovine respiratory disease.

Opportunities now exist to characterize lymphokines fully, isolate the genes that code for them, and clone these genes to obtain sufficient quantities of various lymphocyte hormones for the study of their immune response regulation properties. Scientists already have accomplished these steps with interferon. Current investigations of lymphokines, made possible with recombinant-DNA technology, emphasize the potential for research directed toward molecules that regulate or potentiate the immune response.

The discovery of natural mediators that could decrease animal losses caused by alterations in immunological competence during shipment, weaning, or other periods of stress would be of exceptional value. In these situations, normal management of endemic diseases is hindered by the apparently altered immune response. Stress in livestock and avians often results in decreased reproduction and growth performance, and, at times, even death of the animal. This adds up to very large annual losses that are difficult to quantitate in actual dollars.

The lymphokines, as natural immune modulators, may be extremely beneficial in influencing the immune response. Basic research in this area is directing scientists toward development of natural products that are easily metabolized by the system. These products may ultimately be more effective and economical than synthetic pharmaceuticals.

**Antibodies** The genetic region that codes for antibodies is remarkable; it directs the synthesis of an antibody to virtually any foreign molecule by rearranging the DNA in the immune cell. Intensified investigation of antibodies and the T cell receptor in food animals would particularly apply to ongoing studies of a number of important livestock viral diseases such as bluetongue, malignant catarrhal fever, bovine leukemia, scrapie, pseudorabies, African swine fever, Marek's disease, and avian influenza and leukosis; bacterial diseases such as diarrhea of the newborn and mastitis; rickettsial diseases such as anaplasmosis; and parasitic diseases such as babesiosis.

The greatest problem in combating these diseases is providing early diagnosis so that treatment can be given before economic loss occurs. There is an immediate need for antibody reagents that will clearly distinguish disease-causing pathogens at an early stage of infection. In addition, increased knowledge of T cell functions will provide information about cell-associated immunity and the immune response. Effective vaccines can best be developed when scientists have a clear understanding of the immune response, which varies with the disease-causing organism and the species of animal involved.

Furthermore, these studies must be conducted in food animals. Information cannot be extrapolated directly from humans or laboratory animals. Advances in the study of antibodies and the immune response in these animals will directly benefit the livestock industries and provide additional benefit to medical science.

#### Pathogens and Vectors

Most vaccines consist of the organism that causes the disease, either killed or treated in various ways (attenuated) to reduce its virulence. The immune system responds to the killed or attenuated vaccine by producing antibodies that bind to antigens on the surface of the pathogen, labeling it for attack. The antibodies pro-

duced against the modified pathogen circulate throughout the body and render the animal resistant to a later infection by the live pathogen, thus protecting the animal against the disease.

Because these vaccines contain the entire pathogen and its complete genetic material, there is some risk that attenuated strains may yet be potent enough to actually cause disease. In other cases, however, vaccines consisting of inactivated virus have not stimulated antibody production in the animal, and immunity has not been conferred. In addition, these vaccines are specific for a particular pathogen and generally offer no protection against the variety of subtly different strains that may be present.

Conventional vaccines of denatured, inactivated virus have failed to provide immunity against diseases such as bovine viral diarrhea. Live vaccines have been found not only to be inadequate but also in some instances to have contributed to the spread of disease.

The development of subunit vaccines, which contain only the critical part of the pathogen necessary to stimulate antibody production and not its genetic material, will solve many of the problems presented by conventional vaccines. Scientists working on foot and mouth disease at the ARS Plum Island Animal Disease Center determined the amino acid sequence of the immunogenic protein for a subtype of that virus. They were then able to identify and clone the gene that codes for the viral surface protein and prepare a subunit vaccine for a subtype of the virus. Foot and mouth disease attacks all cloven-hooved animals, and, although it was eradicated from the United States in 1929, outbreaks in other parts of the world and the potential for transmission of the disease are continuing threats to U.S. livestock.

Monoclonal antibodies give scientists the precision to completely define the virus and its strains, and aid in the genetic engineering of effective subunit vaccines. Once perfected, these steps can be applied to other viruses and to bacteria and parasites.

Disease-causing parasites, including both the single-celled protozoans and the many-celled metazoans, are particularly difficult to combat with vaccines, because they have the chameleonlike ability either to alter or to mask their antigens, and thus escape recognition by the antibody. A well-known example of this phenomenon is the African trypanosomes that cause trypanosomiasis, or sleeping sickness, in humans and other animals. During

an infection new antigenic variants are unaffected by the immunity to previous variants. By the time the response to the new antigen reaches effective levels, a still newer variant is being produced. This mechanism keeps the parasite a step ahead of the host's protective response and allows its survival regardless of the effectiveness of the immunity.

A costly example of this type of phenomenon is anaplasmosis, the tickborne rickettsial disease that causes severe anemia and death in cattle. Anaplasmosis-related losses, including death, persistence of the infectious agent in surviving animals, and reduced performance of survivors, cost the U.S. industry \$100 million in 1983. The complexity of the immune mechanism in parasitic diseases renders a disease-control program via vaccine difficult and may call for research directed in a related area--vectors.

Insects and other arthropod vectors not only transmit disease but also serve as reservoirs for pathogens between disease outbreaks. Diseases can often be controlled, however, if the vector can be altered or eliminated. Cloning of specific genes of vectors--gnats, ticks, black flies and mosquitoes--can substantially increase understanding of the transmissibility of a disease agent and aid in its eradication or control.

Genetic manipulations of microbial agents such as bacteria, viruses, protozoa, or fungi may result in the creation or enhancement of agents lethal to the vector. To reduce the necessity of repeated applications to vector-infested areas, a bacterial control agent, for example, would have to be genetically designed to thrive and reproduce toxin-bearing generations in the wide variety of habitats where the vectors are found. One promising use of bacterial control of vectors involves Bacillus thuringiensis, Serotype H-14, and its natural toxins that are deadly to mosquitoes and black flies. To be most effective, B. thuringiensis would have to be adapted to brackish water, pollutants, and other conditions common to mosquito-infested areas.

Genetic studies of the characteristics of vectors should focus on factors that influence vector competence, or vector efficiency--the intrinsic factors and mechanisms that control the ability of insects and other arthropods to carry and transmit disease agents. Barrier systems exist in vectors that prevent a disease agent, such as a virus, from spreading into the different cells and tissues in the vector. This limits or eliminates the

vector's ability to transfer the disease agent. The mechanisms of the barrier systems are not well understood. They appear to be under genetic control and can be expressed in varying degrees within a vector population, thus affecting the epidemiology of the diseases.

#### Disease Control

Extensive studies have established that many diseases can be controlled by a combination of procedures including vaccination, enhancement of the immune response, vector control, diagnosis, and therapy. As discussed previously a better understanding of infectious agents will lead to improved vaccines. Characterization of recognition properties between vectors and the disease agents they transmit will provide clues to control sures. The increasing knowledge about genes that regulate the immune response has already led to the identification of lymphokines and other immune response enhancers. In addition to these areas the further exploitation of monoclonal antibodies and recombinant-DNA technology will improve current methods used to identify and control infectious agents.

Molecular Diagnostics Both monoclonal antibodies and DNA manipulation can be employed to fully characterize and detect pathogens. Monoclonal antibodies that recognize specific antigens can be prepared relatively easily in the laboratory, and pathogen-specific nucleic acid sequences can be identified by restriction enzyme mapping and by RNA or DNA hybridization techniques. The sensitivity and utility of the methods are attested to by their increased use in diagnostic research in humans.

Conventional diagnostic reagents have proven inadequate for numerous diseases. No effective reagents yet exist to diagnose malignant catarrhal fever, a fatal herpesvirus in cattle and sheep. As a consequence, there is currently no effective way to control the disease. Scrapie in sheep, introduced into the United States in 1947, is another critical disease. Scrapie presents a difficult diagnostic problem because of its extended incubation period of up to three years. Diagnostic tests based on monoclonal antibodies are under development for bovine leukosis virus and bluetongue, a viral disease in sheep that is transmitted by gnats.

Monoclonal antibody diagnostic products could be useful for disease control programs both in the United

States and abroad in monitoring levels of disease in a herd as well as in initially detecting disease. Monoclonal antibodies and recombinant-DNA techniques both could be used to identify critical immunogenic components for inclusion in subunit vaccines and as tools to isolate antigens that have the potential for use as effective vaccines.

Subunit vaccines also could be prepared by chemically synthesizing peptides, linear polymers of amino acids. The synthetic peptides would be based on known amino acid sequences of viral surface proteins. Synthetic peptides corresponding to part of one viral surface protein of foot and mouth disease have been shown to protect animals against live foot and mouth disease virus of that type.

Subunit vaccines would provide greater effectiveness with less risk than conventional vaccines and have the potential to be produced economically.

**Therapeutic Agents** The potential exists to use monoclonal antibodies to develop immunotherapeutic agents. In humans, antibodies to specific toxins and pathogens such as antivenin and diphtheria antitoxin are effective. Antibodies administered by mouth or by injection have recently been shown to have a beneficial effect in animals with bovine diarrhea caused by the bacteria Escherichia coli. Monoclonal antibodies increase the precision of specificity of the therapeutic agents used and avoid the problem of injecting extraneous proteins during therapy.

In a number of instances the depression of the immune response results in onset of disease, for instance, in gram negative bacterial septicemia. The use of monoclonal antibodies can potentially prevent or arrest such infections, especially where drug therapy is contra-indicated. The value of such immunotherapy has been recently demonstrated by the successful treatment of neonatal pigs and calves with monoclonal antibodies specific for pili antigens on enterotoxigenic E. coli that cause diarrhea. The whiskerlike pili located on the surface of the bacteria, provide a means of adhesion so that the pathogen can colonize the gut mucosa and produce a high concentration of its toxin. The process is inhibited by administration of the monoclonal antibody.

#### Research Status

The immediate opportunities provided by the newer biological technologies set the stage for relatively

rapid research advances in the area of animal disease. With intensive research effort and use of molecular techniques, safer and more effective vaccines and diagnostic and therapeutic products could be available within a few years.

Recent appointments of new scientists trained in molecular biology at many of the ARS centers are increasing the potential productivity of each of these laboratories. Additional appointments in molecular biology, immunology, genetics, biochemistry, molecular pathology, and computer science will enhance the catalytic effect that these newer technologies can have on research productivity. Excellent examples of this approach are the basic research program in parasitology at the Beltsville Agricultural Research Center in Maryland and the exotic animal disease research program at the Plum Island center in New York, where genetic engineering methods are being exploited to develop a safe vaccine for one type of foot and mouth disease.

The ARS will benefit by focusing research on a number of specific diseases and by concentrating on the full utilization of existing facilities and the acquisition of new equipment. Major benefits will result from an extension of interdisciplinary collaboration that includes not only ARS scientists but also those from university and industrial laboratories. Special attention must be directed toward the following areas:

- Studies of the molecular structure of genes that regulate the immune response, the immune response itself, and the genetic basis of disease susceptibility. These areas could be pursued most efficiently at Ames, Iowa; Beltsville, Maryland; and Clay Center, Nebraska.

- Definition of the molecular organization and antigenic composition of pathogens including viruses, bacteria, and protozoan and metazoan parasites, to be used to develop refined molecular diagnostic tests using monoclonal antibodies. Such studies should be emphasized at the Ames and Plum Island centers.

- Development of the scientific base for subunit vaccine production and the use of other antigenic components for the production of improved vaccines at Ames.

- Development of the scientific expertise to use host-derived immune modulators to enhance the immune response to improved vaccines. These might include lymphokines, such as interferons, interleukins, and others. Ames and Beltsville are appropriate centers to support research in this area.

Several key laboratories around the world are contributing to molecular and other aspects of research in food animal diseases. These include both government-operated and university laboratories.

#### Molecular Basis for Metabolic Regulation

The efficiency of food production by agriculturally important animals is related to the regulation of metabolism during pregnancy, growth, and lactation. The new biotechnology offers versatile approaches to the understanding of physiological processes and the subsequent improvement of metabolic efficiency. Already on the horizon is the promise of increases in efficiency equal to those obtained during the last 20 years using traditional animal breeding and selection programs.

With perhaps the exception of the major hormones governing the reproductive cycle in mammals, little is known of endocrine control in food-producing animals or of the environmental and physiological factors that alter the secretion and clearance rates of hormones. Similarly, the synergisms and antagonisms among hormones and the relationships between hormonal response and cellular receptors are unknown. Progress in understanding endocrine control mechanisms can be accelerated by using recombinant-DNA methods and monoclonal antibodies in combination with such classical techniques as electron microscopy, radioimmunoassay, and cell culture methods. Endogenous chemical mediators as well as their effects on the metabolism and function of different cell types must be identified and characterized. Research focused on understanding the influences of endogenous chemical mediators and on the consumption, digestion, and utilization of nutrients will lead to increased metabolic efficiency in food animals.

#### Characterization of Endogenous Chemical Mediators

Hormones are chemical mediators that coordinate body processes. During pregnancy, for example, hormones promote the uptake and use of nutrients by the gravid uterus and alter the metabolism of maternal tissues to support fetal development. Hormones likely to hold key roles in the regulation of nutrient utilization include prolactin, progesterone, estrogen, placental lactogen,



glucagon, growth hormone, insulin, and corticoids. Undoubtedly, additional hormones will be identified. Already the use of standard cell culture techniques has led to identification of a series of small polypeptide hormones such as somatomedins and epidermal and bone growth factors that may be important regulators of cellular growth.

Once a peptide hormone is identified and purified, it should be possible to produce monoclonal antibodies that are specific for that hormone. Using these antibodies as probes, the location and endogenous levels of the hormone can be determined. Identification and isolation of genes that code for a particular hormone also may be possible using monoclonal antibodies or other techniques. In fact, a peptide hormone with a known amino acid sequence can be synthesized if it is of a manageable size. Sufficient quantities of peptide hormones might be produced in bacterial systems using recombinant-DNA techniques to permit the characterization of their biological importance in food-producing animals. The potential significance is illustrated by the progress in growth hormone research. Recently, recombinantly derived bovine growth hormone has been produced in quantities large enough to administer to test animals. Preliminary results show that injections of bovine growth hormone can cause up to a 40 percent increase in milk production in dairy cows and a marked increase in growth rate in beef animals.

Research in the area of endogenous chemical mediators has tremendous potential for direct applications that will result in significant increases in the efficiency of animal production.

#### Metabolic Control and Function of Cells

An elaborate system exists within the cell to regulate the metabolism of proteins, carbohydrates, and lipids. Although limited, data on food animals have frequently demonstrated critical differences among these species and laboratory animals. For example, the effects of insulin on biochemical pathways and the regulation of lipid synthesis in food animals have been shown to differ significantly from effects in laboratory animals and humans.

Before progress can be made in many facets of cellular metabolism research, the bioregulatory processes must be characterized in key tissues such as muscle, mammary, liver, adipose, bone, and placenta. Important areas of

research include the identification of nutrient and ion transport mechanisms, cellular membrane and organelle roles, and key enzymatic sites of regulation. It is critically important to identify the mechanisms by which extracellular signals, the communication between organs, arrive at an individual cell, bind to it, and then are amplified within the cell to coordinate intracellular biochemical processes. Monoclonal antibodies, affinity chromatography, and nuclear magnetic resonance, which makes use of the absorption of electromagnetic waves to identify receptor structures and characterize biochemical events, provide an unprecedented opportunity to probe the biological processes in cell metabolism. Monoclonal antibodies, for example, because of their elegant specificity, can be used to block specific transport systems, enzymes, and regulatory proteins. This allows for the identification of the key steps regulating both nutrient uptake by the cell and nutrient use in such processes as protein and fat accretion in muscle and adipose tissues.

A clearer understanding of these biological processes will lead to means of manipulating them to achieve greater animal efficiency. For example, a decrease in protein turnover might markedly enhance the efficiency of muscle growth, since the rate of protein degradation is as high as 75 percent the rate of protein synthesis. Similarly, a decrease in fat deposition by adipose tissue would dramatically increase the efficiency of growth. More than 1 billion kilograms of excess fat are trimmed from beef carcasses in the United States annually, representing a billion-dollar loss that is absorbed by producers, processors, and consumers. Research emphasis should be directed toward an understanding of the basic biology that determines the partitioning between tissues such as muscle and adipose.

#### Factors Influencing Intake and Digestion

The performance of an animal is dependent upon the interactions of food consumption, digestion, and absorption. Animal production is dependent upon nutrient supply and therefore upon the appetite of the animal. The control of food intake is coordinated by the central nervous system in response to gut pressure, pattern and quantity of absorbed nutrients, and factors associated with rates of tissue metabolism. Genetically superior animals that have high rates of growth or milk production

are able to consume a much greater than normal quantity of feed. Conversely, loss of appetite exacerbates many of the effects of stress and clinical or subclinical disease states in animals, including humans. Unfortunately the biology of appetite control is not understood well enough to allow manipulation for improved production. Recent advances in high-resolution instrumentation and the use of monoclonal antibodies to identify biological mediators such as gut hormones now provide opportunities to gain new insights into the mechanisms of factors that determine food intake.

There also are distinct differences among ruminant and nonruminant animals that have significant implications for production efficiency. The ability of the ruminant to utilize forages is dependent upon microbial fermentation. Until recently microbial action in the large intestine of nonruminants such as pigs was not fully appreciated in relation to nutrient digestion and absorption. The digestive action of these microorganisms makes possible the uptake by animals of some nutrients in feedstuffs that otherwise would not contribute to the human food supply. Genetic engineering technology makes possible the modification of organisms that might enhance the utilization of nutrients and the nutrient profile of plant materials. Microorganisms engineered to degrade plant lignin, for example, would increase the availability of nutrients from low-quality plant materials.

The metabolic regulation of nutrient utilization for physiological processes such as growth and lactation is complex. Developments in biotechnology offer unique opportunities to identify and manipulate the key controls of metabolic regulation. There can be no doubt that these efforts will lead to tremendous increases in the efficiency of food production in food animals.

#### Research Status

The efficiency of food production by animals is closely related to the regulation of metabolism during pregnancy, growth, lactation, and egg production. The new biological techniques, as tools, provide tremendous opportunities to understand physiological processes and to apply this knowledge to improved metabolic efficiency.

The area of metabolic research within the ARS is significantly understaffed relative to its importance. An increasing number of scientists must direct their efforts

to the study of growth, lactation, and reproduction in dairy and beef cattle, sheep, pigs, chickens, and turkeys. The opportunity to create the appropriate critical mass of scientists for effective research in the basic biology of food animals will be lost unless there is considerable expansion or consolidation of research groups at the Beltsville Agricultural Research Center, the research center at Athens, Georgia; and the U.S. Meat Animal Research Center at Clay Center, Nebraska.

The new biology methods offer unprecedented opportunities to probe the biological processes of cellular metabolism. The committee recommends that the Beltsville laboratories intensify their focus on basic cell biology research, capitalizing on the strong basic biomedical research programs in metabolic regulation at the neighboring National Institutes of Health.

In addition, the ARS can further improve studies of metabolic regulation by establishing carefully focused programs at Clay Center in embryo survival, the genetic bases of disease and growth efficiency, systems modeling, and the introduction of new germ plasm.

More specifically the ARS should:

- Identify, isolate, and characterize specific endogenous chemical mediators involved in organ-organ and cell-cell communication;
- Develop fundamental knowledge of intracellular regulation of metabolism and functional interrelationships between organelles and other cellular components;
- Delineate the response mechanisms involved in the translation of extracellular signals into intracellular biochemical events;
- Identify interrelationships between feedstuffs, microbial fermentation, and nutrient availability in the digestive tract;
- Characterize mechanisms and factors associated with the efficiency of nutrient absorption from the digestive tract; and
- Using this new knowledge, develop means to manipulate these key control systems in specific tissues such as muscle, adipose, and bone, and thereby increase the efficiency of animal production.

Currently only a very few small laboratory groups are studying endogenous chemical regulators and cellular metabolism.

### Developmental Biology and Reproduction

Animals expend an enormous amount of energy to reproduce themselves, and successful reproduction obviously is necessary to obtain sufficient animals for production purposes. Modest improvements in reproductive efficiency of livestock in this country would be worth millions of dollars annually. New biology methods offer special opportunities to increase reproduction, which in turn should result in marked gains in productive efficiency. In addition, new tools have become available to study and modify differentiation. These tools will be of great importance in all areas of biology.

#### Differentiation

At the two-cell stage of mammalian embryonic development, each cell is equivalent and totipotent: Each cell can develop into an adult organism, resulting in identical twins. As embryonic development proceeds, cells differentiate into specialized tissues, such as muscle, bone, and nerves; and totipotency is lost. No longer can a fetus be obtained from a differentiated cell such as a nerve or muscle cell.

The genetic and molecular processes by which embryonic cells become specialized and then irreversibly become fixed as specific cell types are the basis for the unanswered questions of developmental biology. Is differentiation mediated primarily by changes in cytoplasm that regulate DNA or is there some fundamental change in the DNA? What is the nature of the change in cytoplasm or DNA, and is it reversible? Is the genetic information obtained via the ovum equivalent to that obtained via the sperm, and if not, how does it differ? What are the molecular mechanisms of cell-cell interaction during differentiation?

Procedures incorporating nuclear transplantation and recombinant-DNA technology now provide the tools necessary to address these kinds of questions. Information gained in attempts to answer these questions should be useful for turning genes on and off in both cell lines and adult tissue. Just as medical researchers have switched on the gene for fetal hemoglobin production in humans with sickle cell disease to compensate for the production of defective hemoglobin by the adult gene, so too might the gene for double muscling in cattle be transferred and switched on in market animals.

The double muscling mutation occurs in a number of breeds, such as the Belgian Blue; however, the animals often do not reproduce well. Manipulation of the gene in beef cattle at the appropriate stages of growth could greatly enhance productive efficiency.

Understanding differentiation is absolutely fundamental in nearly every area of biology; all cells, whether adipose cells or muscle cells, emerge from undifferentiated cells. Basic studies on differentiation should be a high priority, particularly because the tools now available will allow rapid progress.

#### In Vitro Manipulation of Gametes and Embryos

It is possible to collect embryos from females, culture them in vitro, freeze and store them indefinitely in liquid nitrogen, then bring them back to activity, sex them, and transfer them back into the reproductive tract of recipients to obtain normal offspring. Individual embryos can be divided into two microsurgically, which results in identical twins. Division into three or four parts produces identical triplets, but the success rate is lower than for twins.

These techniques are useful in increasing the reproductive rates of females, much like artificial insemination has been used in males. It is unlikely, however, that embryo transfer will replace artificial insemination on a routine production basis within the next decade, simply because of the ease and results gained using artificial insemination. It is not unusual to obtain 10,000 offspring from one bull in a single year by artificial insemination; embryo transfer might provide 15 offspring from a single cow in the same time period.

Only 4 percent of the U.S. beef herd is artificially inseminated, but the technique is used in about 70 percent of the national dairy herd where the specific trait of milk production is passed successfully to subsequent generations.

In the dairy industry, however, production of bull calves by embryo transfer may provide an efficient means of amplifying the genes of the best cows through their sons. Additional applications of this technology include the intercontinental transport of germ plasma via embryos economically and with less risk of spreading disease than with transport of animals or semen. The use of embryo transfer will likely increase dramatically, particularly

in animals of high value, as nonsurgical methods quickly replace surgical procedures and reduce the cost of equipment and personnel.

Embryo transfer techniques also are important for basic research. For example, when genetic variation must be controlled precisely in an experiment, manufactured identical twins or multiplets can be used. Maternal effects on development can be investigated by placing half an embryo into one kind of female and the matching identical half into another.

Further research, however, must be conducted to improve some of these methods for use in domestic animals. In vitro fertilization techniques have been particularly successful in the rabbit, mouse, and human, but work poorly in food animal species. Embryos cannot be cultured in vitro for longer than a day without damage, and cryopreservation kills one-fourth of the embryos.

These problems are related both to species differences and to the specific technical procedures required for various animals. In the cow, for example, embryo transfer is a relatively simple and successful procedure, but in vitro fertilization attempts have failed. For a yet unknown reason, bovine embryos survive cryopreservation at a much higher rate than do pig embryos. In general, sperm are much easier to freeze than liver or embryo cells.

There is an urgent need to conduct fundamental research in areas such as cryopreservation, in vitro fertilization, and nutrient requirements of embryos. These technologies are essential for the conduct of progressive research. They would improve methods of germ plasm preservation and provide insights into problems such as fertilization failure and embryonic death in vivo.

#### Addition of Genetic Information to Embryos

The ability to obtain embryos by in vitro fertilization or to remove them from the female reproductive tract temporarily for various procedures is useful in a variety of genetic manipulations. For example, it is possible to inject genes into the pronuclei of a one-cell embryo so that the genes are duplicated automatically each time the cells divide. In this way each of the billions of cells in the resulting offspring contains the introduced gene. When rat growth hormone genes were introduced into mouse embryos by this method, the extra gene copies greatly

increased the growth rate and subsequently, the size of mice.

Using gene transfer techniques, useful genes could be transferred from one species into another, an accomplishment that would be impossible using selective mating. An example of this manipulation is the transfer of the Booroola fecundity gene from sheep to cattle. Sheep carrying this gene release four or five eggs at ovulation rather than one, a circumstance that would generally not be favored by natural selection but one that scientists might exploit to increase reproductive productivity.

Cell fusion techniques might be employed to transfer genetic material from a somatic cell into a fertilized single-cell embryo for cloning. Viral vectors also might be used as another method of introducing genetic material--retroviruses may be ideal for this purpose. It should be possible to introduce new genes into the fertilizing sperm or the embryo itself by direct uptake of DNA from the bathing medium.

Another useful research tool is the literal mixing of cells from different embryos to form chimeras. Resulting animals possess cells of different genetic composition in different parts of the body. Similar procedures have been used to create a goat-sheep chimera, known as the geep, which presents an opportunity for the study of the relationship between cells of different species during development.

Clearly, these are compelling tools that can be used to answer the fundamental questions of animal reproduction. There is an immediate need to develop these emerging technologies for application to livestock for the future benefit of animal production.

#### Reproductive Efficiency

Reproductive success is central to efficient animal production. The increasing economic values of growth rate efficiency and resistance to disease are magnified by improvements in the reproduction rate. Less than 70 percent of adult female farm animals produce live young in any given breeding season. Barriers to reproductive efficiency include production of nonviable gametes, fertilization failure, embryonic mortality, and losses at birth and in the first few weeks of extrauterine life. Two of these areas, oogenesis, the production and maturation of the egg, and embryonic mortality, are



especially appropriate for study with the newly available biotechnologies and will become particularly pertinent as embryo transfer technology becomes more widely adopted.

Food animals have approximately 100,000 ova in their ovaries at birth; no new ova are made after this time. In the course of a reproductive lifetime, several hundred eggs may be ovulated. More than 90 percent, however, degenerate via a process called atresia. Virtually nothing is known about control of atresia. New biotechnologies, such as cell fusion, could be used to study the normality of the genome, thus providing a better understanding of the nature of this process. Such investigations would increase fundamental knowledge and might also result in the discovery of practical ways of harvesting large quantities of ova.

Embryonic wastage is an even more serious problem; about 25 percent of all conceptions in food animals result in early embryonic death. Some embryonic wastage may be due to infectious diseases, and where the cause is unknown, pregnancy may be terminated because the embryos are genetically abnormal or because of an abnormal uterine environment.

The new biotechnologies such as embryo transfer provide a means of understanding the problem. It is entirely appropriate that agricultural research be expanded in this area, especially since species differences in reproductive processes necessitate the study of food animals themselves.

#### Research Status

The new biology methods can greatly enhance the understanding of reproduction and the study and modification of differentiation, important not only to the agricultural sciences but to all areas of biology.

To establish a leadership stance in developmental and reproductive biology, the ARS must bring clear focus and depth to its existing programs by consolidating the programs at a number of centers and then expanding research efforts, primarily at two centers, Beltsville and Clay Center.

Major areas of research emphasis should include:

- In vitro manipulation of gametes and embryos, specifically the maturation of oocytes, in vitro fertilization, and in vitro culture techniques;
- Addition of genetic information to gametes and embryos;

- Study of the genome at the molecular level; and
- Study of oogenesis and embryonic mortality.

The committee also recommends that the ARS establish a food animal gene bank that would assist the animal science research community by facilitating, coordinating, and fostering the storage and maintenance of DNA libraries, gene transfer vectors, and probes. This service might be analogous to tissue culture cell banks established and maintained for the biomedical research community, including the registry of cell lines at the American Type Culture Collection, Rockville, Maryland; the Human Genetic Mutant Cell Repository maintained by the Institute for Medical Research, Camden, New Jersey; and the National Cancer Institute's Frozen Tumor Bank maintained at the Frederick Cancer Research Facility in Frederick, Maryland.

There are several substantial laboratory groups in institutions studying animal differentiation, in vitro manipulation, addition of genetic information to gametes and embryos, and reproductive efficiency. With the exception of reproductive efficiency, most of this work has not been applied to food animals.

## 4 Plant Science

There have been remarkable advances in the molecular understanding of a few of the key processes in plants during the past decade. An important component of these advancements has been the application of new technologies for isolating, cloning, and characterizing genes. The ability to apply these techniques is based on fundamental knowledge in physiology and biochemistry that has accumulated over the years. Their application has opened new frontiers in the study of plant growth and development.

Molecular genetic approaches have been applied to nearly 50 plant genes, primarily those associated with seed storage proteins, chloroplasts, photosynthesis, and biological nitrogen fixation. Notably, a number of the genes that code for important enzymes in photosynthesis and nitrogen fixation have been identified, cloned, and sequenced. Several of the genes for storage proteins in crop plants have been cloned and characterized. In addition, the ability to regulate the expression of genes controlling the biosynthesis of the key enzymes in photosynthetic carbon dioxide fixation is under active study. Studies are also being initiated to clone the genes for phytochrome as well as those for some of the known plant hormones. Comparable progress is needed in other areas of plant science research.

A lack of basic information on the biochemistry of many metabolic and regulatory steps is delaying progress in using molecular genetics to establish the mechanisms employed in controlling plant growth. This chapter suggests ways of strengthening research that emphasizes integration of traditional biochemical and physiological

research with molecular genetic approaches. Three general areas of research, all of which are basic to plant growth, are discussed: (1) the interaction of carbon and nitrogen metabolism in supporting optimal plant growth; (2) the role of plant hormones and phytochrome in regulating plant growth and development; (3) and the limitations to growth imposed by physicochemical stresses such as cold, heat, drought, and salinity.

### Carbon and Nitrogen Input for Plant Growth

Both the carbon and nitrogen that are essential components for all forms of life ultimately cycle through the atmosphere. Plants take up these elements, in the form of carbon dioxide and nitrogen gas, from the atmosphere via photosynthesis and nitrogen fixation. Both processes involve reduction reactions, which require energy. Solar energy drives the photosynthetic reduction of carbon dioxide into sugars. The chemical energy stored in these products of photosynthesis is then used for nitrogen fixation. The relative availability of these two key constituents, reduced carbon and reduced nitrogen, can greatly regulate plant growth.

### Photosynthesis

Photosynthesis encompasses the most important reactions on earth; life depends directly upon solar energy captured and stored photosynthetically. Not only do plants, through photosynthesis, reduce carbon dioxide to the food and fuel products that sustain life, but photosynthesis also produces the oxygen required to reoxidize these products to release their energy.

Light capture, carbon dioxide fixation, and oxygen evolution were recognized as components of the photosynthetic process more than 200 years ago. These partial reactions have now been described in considerable detail. Initial light capture and energy conversion occurs within a few nanoseconds after a photon is absorbed by chlorophyll. Subsequent electron and proton transfers, occurring within a few milliseconds, generate chemical energy in the form of adenosine triphosphate (ATP) and reduced pyridine nucleotide (NADPH). These compounds in turn power the reduction of carbon dioxide.

### Chloroplast Functions

The chloroplast is a remarkable organelle. It contains all the units essential for photosynthesis--the light-gathering pigments, the membrane components and cofactors that mediate electron transfer, the enzymes involved in ATP and NADPH production, the enzymes for carboxylation and reduction of substrates, and the system that liberates oxygen from water.

Strong oxidizing and reducing agents are maintained in close proximity, but their interactions are controlled. For example, the chlorophylls that capture light and drive the energy-coupling steps of photophosphorylation (the process that produces ATP) as well as pyridine nucleotide reduction are organized within the thylakoid membranes in the chloroplast. The enzymes that catalyze the reduction of carbon dioxide for the synthesis of phosphorylated sugar intermediates occur in the stroma surrounding these membranes.

Chloroplasts contain DNA, ribosomes, and the other components needed for protein synthesis. Major advances in identifying the genes that encode structural components of the chloroplast have been made in the last 10 years, largely through integrated studies using genetics, molecular biology, and biochemical analysis. Chloroplast DNA accounts for only about 10 percent of the genetic information needed for chloroplast structure and function. Most of the structural proteins of the chloroplast are encoded in the DNA of the cell nucleus, synthesized in the cytoplasm, and then imported into the chloroplast. The information now available concerning chloroplast inheritance is spawning research toward practical applications. Investigations include manipulating chloroplast genes that confer selective resistance against specific herbicides in crop plants and designing genes that produce the enzymes involved in carbon dioxide fixation to increase the overall efficiency of the carboxylation reaction.

### Carbon Fixation

The path of carbon in photosynthesis has been clearly defined. Carbon dioxide is fixed to yield the three-carbon molecule phosphoglyceric acid, which is then converted to sugars. This process characterizes the so-called  $C_3$  plants. In other plants, atmospheric carbon

dioxide is initially fixed to yield a four-carbon molecule that is translocated to neighboring cells where it is decarboxylated to give up the carbon dioxide. These are known as  $C_4$  plants. This released carbon dioxide is subsequently fixed to yield phosphoglyceric acid via the  $C_3$  pathway.

The formation of phosphoglyceric acid in both  $C_3$  and  $C_4$  plants is accomplished by the enzyme ribulose-1,5-bisphosphate carboxylase-oxygenase, often called Rubisco. It can react with either carbon dioxide to carboxylate ribulose-1,5-bisphosphate to two molecules of phosphoglyceric acid or with oxygen to oxidize ribulose-1,5-bisphosphate to one molecule of phosphoglyceric acid plus the two-carbon molecule, called phosphoglycolic acid, which is involved in photorespiration. This oxidation reaction is wasteful since energy must be consumed to resynthesize the ribulose-1,5-bisphosphate. It would be advantageous, therefore, to cause Rubisco to decrease or lose its oxidative reaction function. Experimental elevation of carbon dioxide in the air produces major increases in yield of most field crops by increasing the carboxylation reaction. Although this technique demonstrates great potential, carbon dioxide enhancement of large areas is impractical.

Rubisco is inefficient in catalyzing the carboxylation reaction. Attempts have been made to improve its efficiency, but the changes induced by genetic manipulation using both mutational selection and site-specific changes in the DNA sequence have only decreased its activity. In addition, attempts to specifically inhibit the oxygenase function without disturbing the carboxylase have been unsuccessful. If the catalytic sites are different, theoretically the oxygenase activity of Rubisco could be blocked. Selective pressures to accelerate the activity of Rubisco have existed for millions of years; it is hardly remarkable that a decade of research has not brought improvement in the carboxylation reaction, which theoretically might be possible.

The problems in bioengineering an improved Rubisco focus on the fact that it is a large enzyme consisting of eight small subunits with molecular weights of 14,000 each and eight large subunits with molecular weights of 56,000 each. The larger subunits are coded for by chloroplast genes, and the small units are coded for by nuclear genes.

The amino acid sequences of the large and small subunits of Rubisco have been completed for some plants.

Research is focusing, in particular, on the large subunit which contains the catalytic sites for carboxylation and oxygenation. Several laboratories are concentrating on genetically engineering changes in the chloroplast genes that encode for the large subunits. Their objective is to locate a form of this enzyme that has an increased overall activity or a selectively decreased oxygenase activity. A great technical limitation to this work exists: There is no gene transfer system yet available for inserting recombinant genes into chloroplasts.

### Photosynthetic Efficiency

On the average, only one quarter of 1 percent of the radiant energy reaching the earth is captured by photosynthetic organisms. This largely is a measure of the density of photosynthetic plants on the earth's surface and the efficiency with which they can absorb light and photosynthesize throughout all the seasons. In contrast, a highly efficient  $C_4$  plant, such as corn, may utilize 5 percent of incident radiant energy during its most rapid period of growth.

What is the potential efficiency of photosynthesis? One answer is provided by measurements of quantum efficiency. Efficiencies of one carbon dioxide molecule fixed per 8 to 10 quanta absorbed have been measured using single-cell algae. These efficiencies are high, in terms of conversion of incident light, and probably can only be achieved under the optimal experimental conditions--very low light intensities in comparison to average sunlight. For this and other reasons, it is impractical to extrapolate these measurements to field crops.

Many factors such as the developmental stage of the plant and the presence of biological and physicochemical stresses can reduce photosynthetic efficiency. In addition, each step in the photosynthetic process, from the absorption of light energy to the conversion and storage of energy in the synthesis of sugar molecules, can be affected differently by various limiting factors. Thus, to improve overall photosynthetic efficiency, researchers must first understand the steps in photosynthesis and the factors limiting their efficiency.

Although it is doubtful that quantum efficiency in the field can approach that achieved with algae in the laboratory, record yields in field plots are a reasonable

goal. A record corn yield in the United States can be more than 300 bushels per acre, compared with an average yield of about 100 bushels per acre. Comparisons indicate that full photosynthetic growth potential is seldom realized.

How can photosynthetic efficiency be improved? One possible approach is suggested by the difference in photosynthetic efficiency between  $C_3$  and  $C_4$  plants. Major crops, such as wheat, rice, and the seed legumes, are  $C_3$  plants. It may be possible to convert them to a  $C_4$ -type metabolism.

$C_4$  plants are more efficient, primarily because the  $C_4$  pathway serves as a metabolic carbon dioxide pump that raises the carbon dioxide concentration, by ribulose-1,5-bisphosphate carboxylase-oxygenase, at the site of carbon dioxide fixation. This increases the rate of carboxylation and at the same time suppresses the rate of oxygenation. This process is carried out at a level near carbon dioxide saturation, resulting in an enhanced rate of net photosynthesis.

There are significant anatomical differences between the leaves of  $C_3$  and  $C_4$  plants. Specialized bundle sheath cells in  $C_4$  plants contain many chloroplasts;  $C_3$  plants have few if any chloroplasts in their bundle sheath cells. It is in the bundle sheath cells of  $C_4$  plants that the carbon dioxide concentration is elevated and increases the rate of carboxylation by ribulose-1,5-bisphosphate carboxylase-oxygenase.  $C_4$  plants may have evolved from  $C_3$  plants.

Nature has provided some plants with intermediate anatomical and biochemical properties. These plants serve as encouraging models. They include the composite Flaveria and the grasses Panicum and Neurachne. These intermediate plants are tools useful in studying the inheritance and relative advantages of photosynthetic efficiency in the  $C_3$  and  $C_4$  pathways, under different limiting factors. Some of the leading research on photosynthesis in  $C_3$  and  $C_4$  plants has been conducted in ARS laboratories.

The photosynthetic potential of a plant cannot be achieved if its growth is limited by physicochemical stresses or by nutrient deficiencies. Efforts to improve photosynthetic efficiency can be enhanced by research focused on resistance to physicochemical stress and utilization of nutrients from the soil.

Although outlines of the basic steps of photosynthesis appear clear, virtually every aspect of this complex



process requires continued investigation. A thorough understanding of the basic mechanisms of photosynthesis may reveal new information that will permit researchers to increase photosynthetic efficiency and productivity and direct it toward the generation of the most desirable plant products.

#### Harvest Index

The most effective way to improve the harvest index (the ratio of harvested part of the plant to total plant) may be to improve the accumulation of photosynthate in the desired plant part.

Traditional breeding methods have selected for an improved harvest index in many crops and have led to substantial gains in crop yields. Such successes through plant selection have been achieved in the absence of a clear understanding of the factors under genetic as well as environmental control that determine crop yield. Additional improvements in yield and harvest index may depend on a full understanding of these factors and their interactions at the molecular and genetic level. With this information, scientists may now be able to take advantage of recombinant gene transfer methods to further improve crop quality and yields.

Plants have often been selected as crops based on their parts that accumulate the products of photosynthesis. Plants with lush vegetative growth are used for feed or fodder while they are undergoing rapid photosynthetic growth. Alternatively, plants that deposit their photosynthate in stems, roots, fruits, or seeds may be selected for these attributes and harvested after their storage organs have achieved maximal size. In these plants the investigator attempts to redirect photosynthate to the portion of the plant that will be used for feed or food. Researchers are currently attempting to improve the harvest index in soybean, for example.

Before harvest the soybean plant undergoes senescence and mobilizes a high percentage of its nitrogen from roots, nodules, stems, and leaves for deposition in the seeds as protein. In a sense the plant destroys itself to produce a viable, energy-rich seed to preserve the plant line for the next season.

If the soybean plant's delicate control mechanisms can be manipulated to prolong the period of active photosynthesis in the plant without destroying its ability to

go through senescence at the proper time, the harvest of seed will be greater. Foliar application of plant growth substances or nutrients can artificially prolong active vegetative growth. Subsequently, total crop yield may be increased. Integrated research programs that focus on both photosynthesis and developmental biology will contribute to a future understanding of the factors that link photosynthetic productivity and storage mobilization capacities.

Photosynthesis in the chloroplast produces hexoses and hexose phosphates that are either converted to and immobilized as chloroplast starch or converted to sucrose. Sucrose is the major form of carbohydrate transported from the site of photosynthesis in the leaf to other parts of the plant. It is readily converted to starch and other storage products in seeds and storage organs. Control of the transfer of sucrose to various parts of the plant and optimization of deposition of carbohydrate, protein, and fat reserves in seeds and storage organs determines the harvestable yield of a crop. Using empirical methods in plant breeding and selection, researchers have successfully increased the harvest index of many plants. Little is known, however, about the processes regulating the translocation and metabolism of photosynthetically fixed carbon.

Species vary in their rates of accumulation of starch and sucrose in leaves. Wheat, barley, and spinach accumulate more sucrose than starch in leaf mesophyll cells in contrast to species such as peanuts, soybeans, and tobacco, which accumulate more starch than sucrose. Studies on the enzymatic steps involved in the biosynthesis of starch and sucrose indicate that inorganic phosphate and triose-phosphate have profound effects on regulating the rates of these interconnected biosynthetic pathways. Research focusing on a full understanding of the regulation of the storage and transport of carbohydrates has been modest.

The partitioning of photosynthate is a major factor determining harvest index. Research on the metabolism of the hexose products of photosynthesis and the regulation of their conversion to carbohydrate, protein, and lipid storage products should be increased.

#### Nitrogen Metabolism

Nitrogen is a key element required by plants, and it is commonly the limiting element in plant productivity.

The world capacity for commercial fixation of nitrogen is about 60 million metric tons per year, the bulk of which is used as fertilizer. Dependence on this source for agricultural use has created problems. High cost precludes its use in many areas; natural gas as the feedstock for chemical fixation is a limited, nonrenewable resource that will increase in cost; and the use of too much fertilizer may be accompanied by excessive losses through leaching, erosion, and denitrification. Leaching into water supplies may raise nitrate concentrations to harmful levels.

When biological fixation is substituted for chemical fixation of nitrogen, the energy of sunlight is substituted for the energy of natural gas. Sunlight is captured through photosynthesis, and its use preserves fossil fuels. The nitrogen fixed biologically in the root nodules of leguminous plants is quickly assimilated into organic nitrogen compounds in the plant and is subject to very low levels of leaching and denitrification. Although some major seed and forage legumes such as soybeans and alfalfa take advantage of biological nitrogen fixation, there is potential for improving and extending the advantages of biological nitrogen fixation to other crops.

#### Biological Nitrogen Fixation

The association between leguminous plants and their root nodule bacteria is the preeminent system for biological nitrogen fixation (BNF) in agricultural crop plants. There are, in addition, certain free-living bacteria and bacteria in loose association with plants whose nitrogen-fixing capabilities warrant further investigation.

Substantial advances have been made in studies of the biochemistry and genetics of nitrogen fixation. The enzymes and the electron transfer sequence involved in the steps that reduce nitrogen gas to ammonium can be described in some detail. Study of the genetics of the nitrogenase system in the free-living, nitrogen-fixing bacterium Klebsiella pneumoniae has shown that 17 genes are involved. The function of most of these genes has been defined. Now it is necessary to establish comparable detailed information on the genetics of nitrogenase in other nitrogen-fixing organisms, including the symbiotic bacteria Rhizobium spp., blue-green algal species, photosynthetic bacterial species, and the azotobacter and the clostridia.

In symbiotic biological nitrogen fixation, detailed information on the contribution of both the bacterium and the plant must be determined. For example, studies of the rhizobia-legume association have shown that the genetic information for production of the globin in hemoglobin, found in the leguminous nodules, is contributed by the plant. Scientists may eventually manipulate genes to improve nitrogen fixation or to introduce it into other bacteria or higher plants not now capable of fixing nitrogen. A thorough understanding of the genetic systems of both the bacterium and the plant will enhance the chances of success in transferring genetic elements.

There are marked differences in the effectiveness of symbiotic nitrogen-fixing systems, but the characteristics governing good or poor associations have not been defined. Until these factors are defined, genetic manipulations will remain empirical. In addition, symbiotic nitrogen-fixing systems require large amounts of photosynthate--10 to 12 grams of photosynthate are utilized in fixing 1 gram of nitrogen. Decreasing this energy requirement is a major research challenge.

Nitrogen-fixing systems dissipate 25 percent or more of their energy in producing hydrogen rather than in reducing nitrogen. Hydrogen production is apparently inherent in nitrogenase action. The only way known currently to decrease this energy loss is to recycle the hydrogen to recapture its energy. Oxidation of hydrogen via a hydrogenase enzyme can be coupled to ATP formation and to reductant formation. ATP and reductant can then be used to support nitrogenase activity. The gene for hydrogenase, hup<sup>+</sup>, has been transferred to Rhizobium japonicum. Soybeans inoculated with hup<sup>+</sup> rhizobia produce higher yields of protein than those infected with comparable hup<sup>-</sup> rhizobia, which lack this enzyme. Similar improvements through manipulation of other genes or modification of the nitrogenase genes for increased efficiency can be made when other factors limiting nitrogenase activity are defined.

#### Improving Symbiotic Nitrogen Fixation

About 85 percent of legume inoculant used in the United States is applied to soybeans. Indigenous rhizobia are so dominant in most soybean fields, however, that improved rhizobia strains that are added to the soil

do not compete effectively in the process of nodulation. To improve legume-bacterial symbiosis, the competitiveness of added, improved rhizobia strains must be increased. A superior nitrogen-fixing strain developed under controlled conditions in the greenhouse will be of little use in the field unless it can form nodules on the soybean plants in competition with indigenous bacteria. Processes such as primary attraction, binding, and infection may be important aspects of this competition. Such processes may be influenced by special glycoprotein molecules, (called lectins) on the root surface, but this must be established clearly and it must be controlled.

Mutants of rhizobia have been produced that appear to enhance the early growth of soybeans. This optimal growth, however, has not been maintained until harvest. Improvements under field conditions are of economic significance; however, few have been verified.

Host Plant Improvement Carbon and nitrogen metabolism share an intimate relationship. Biological nitrogen fixation requires great amounts of energy, supplied primarily by photosynthesis. Progress in the understanding of photosynthesis has been impressive, but further research is needed to define its interactions with major limiting factors in plant growth. With advances in experimental techniques and a better understanding of the fundamental metabolic steps in both photosynthesis and biological nitrogen fixation, researchers are better equipped to study the feedback relationships between these two processes. An improved understanding of the interactions among nitrogen metabolism, photosynthetic carbon fixation, and the distribution of fixed carbon throughout the plant will contribute to eventual increases in the harvest index.

Recent studies suggest that transformations of carbon compounds at the site of nitrogen fixation in the plant may be important in nitrogen fixation. Research conducted by ARS scientists has demonstrated the ability of root systems of certain leguminous plants to fix carbon dioxide. These transformations may be a part of the conversion of photosynthate to compounds especially useful as acceptors for newly fixed nitrogen.

The transfer of the genes for nitrogen fixation to nonleguminous plants, such as corn, is appealing and should be studied on a long-term basis. Genes for nitrogen fixation have been transferred from the free-living bacterium Klebsiella pneumoniae to the bacterium

Escharichia coli, and were expressed. These genes have also been transferred to yeast, a eukaryote, but were not expressed.

Transfer and expression in a higher plant is difficult to achieve. The plant, in addition to receiving the nitrogenase genes, must be able to supply the large amount of energy in the form of ATP and reduced pyridine nucleotides required for nitrogen fixation. The plant also must furnish a means to protect nitrogenase against inactivation by oxygen. While the successful transfer of biological nitrogen fixation properties to other crop plants could lead to savings in nitrogen fertilizer costs, the high energy needed to fuel this process may entail a loss in yield relative to that when fixed nitrogen is supplied. Long-term research will be needed to successfully transfer nitrogen fixation to corn and other crop plants.

The thousands of species of nitrogen-fixing leguminous plants such as acacias, leucaena, and winged beans are underexploited as sources of food, fiber, and fuel. These plants should be studied in more detail. Certain nitrogen-fixing nonleguminous plants have great potential for the production of fuel wood on deficient soils. Pressures on fuel wood are increasing worldwide; alder, casuarina, and other comparable nitrogen-fixing plants should be investigated as alternatives to other woody species.

#### Other Aspects of Nitrogen Metabolism

Essential Amino Acids A more complete knowledge of genetic control of the synthesis of storage proteins in plants could lead to development of plant products with improved nutritional value for consumption by humans and food animals. Research on the storage proteins in corn and soybean has received particular emphasis. The genetics governing the production of zein, the corn storage protein, have been defined. Further improvements in the amino acid balance of zein may be possible through genetic manipulation. Comparable work on the storage proteins of food and feed legumes could improve their nutritional value.

The ARS research programs have been contributing effectively to this work on the genetic control of seed protein synthesis.

**Nitrates and Nitrites** Increased levels of nitrates and nitrites are appearing in drinking water supplies. There is concern that the heavy use of nitrogen fertilizers will contribute to this problem. Nitrate is reduced to nitrite in the intestinal tract. Absorbed nitrite complexes with hemoglobin, effectively reducing the oxygen-carrying capacity of the blood. This can be particularly serious in young children. There is apparently less awareness of nitrates introduced into the food supply through ingestion of vegetables, despite the fact that most people take in considerably more nitrates with vegetables than with their drinking water. The influence of cultivar and cultural methods on the accumulation of nitrate in common vegetables should be investigated. Development of plant varieties that accumulate less nitrate may be feasible. In addition, the use of slow release urea fertilizer should decrease the nitrate available for uptake by plants.

**Fertilizer Nitrogen Losses** Nitrogenous fertilizers are expensive; it is important that they be used efficiently. Customarily, less than half the nitrogen fertilizer added to the soil is incorporated into plant products. Nitrogen added as anhydrous ammonia or ammonium salts is effectively tied up by the mineral and organic components of the soil in complexes that are relatively water insoluble. But when nitrogen is converted to nitrate and nitrite by nitrification, it is subject to leaching. Nitrification can be inhibited by commercial agents, such as N-Serve, so that loss of nitrogen by leaching is decreased. By blocking the formation of nitrate and nitrite the process of denitrification is likewise inhibited. Slow-release fertilizers will nourish the plants with reduced losses as will additions of fertilizer at intervals during the growing season. In special instances, foliar application of nitrogenous fertilizers is efficient and practical.

#### **Research Status**

An improved understanding of photosynthesis and biological nitrogen fixation has been achieved through steady, long-term research that has included the application of new experimental methods. These methods, including techniques to isolate, clone, and characterize genes, have provided new insights into each step in these processes. Research must be continued and broadened to

achieve an understanding of the feedback relationships between photosynthesis and nitrogen fixation and, thereby, determine how they influence total plant productivity.

A sustained program that advances fundamental knowledge of carbon and nitrogen metabolism in plants can result in significantly increased crop productivity and lowered costs. The ARS is in an excellent position to establish long-term goals and to give long-term support to multidisciplinary investigation of carbon and nitrogen uptake. The ARS, for example, could expand its efforts to become a major contributor to information on nitrogen metabolism in plants. Emphasis should be placed on genetics, enzymology, leguminous plant associations, efficient utilization of fixed nitrogen, and development of alternative systems for nitrogen fixation.

It is essential that the key processes that determine yield and quality in crops be understood at the molecular level. Only then can researchers take advantage of new techniques to manipulate genetic and chemical regulatory steps that favorably influence these processes. Future ARS research, with emphasis at the molecular level, should include studies of the following:

- The oxygenase and carboxylase properties of the key photosynthetic enzyme, ribulose-1,5-bisphosphate carboxylase-oxygenase, to identify ways to modify the enzyme to improve the overall efficiency of photosynthesis;
- Metabolic and anatomical properties of C<sub>4</sub>-type photosynthetic plants to explore possible transfer of these properties into less photosynthetically efficient C<sub>3</sub> plants;
- Chloroplast membranes and the light reactions of photosynthesis to identify opportunities for improving photosynthetic efficiency and to gain an understanding of the mechanism of action of herbicides that act on the photosynthetic systems;
- Factors influencing chloroplast development and senescence, with special attention to the role of nitrogen levels;
- Genetic determinants controlling the partitioning of photosynthate between the harvested and nonharvested part of the plant, including traits that determine the composition of seeds and other storage organs;
- Nitrogen-fixing systems, including nonsymbiotic prokaryotes such as the azotobacter and blue-green algae, that may lead to incorporation of functioning nitrogenase genes directly into cells of crop plants; and



- Symbiotic nitrogen fixation systems to improve the process in leguminous crops and possibly extend it to nonleguminous crops.

### Regulation of Plant Growth and Development

Current knowledge of the morphological and metabolic changes that occur during the life of a plant, from the germinating seed of one generation to the seed of the next, is primarily descriptive. Only five classes of plant hormones, or growth-regulating substances, and two photomorphogenic pigment systems have been implicated as principal modulators in plant development. Two factors make research on these plant development regulators extremely difficult: (1) they are active in low concentrations, and (2) many developmental steps are orchestrated by the simultaneous effects of several of these regulators.

Much of what is known about the substances that regulate plant development centers on the five classes of plant hormones: auxins, gibberellins, cytokinins, abscisins, and ethylene and the photomorphogenic, light-capturing pigment called phytochrome. The second photomorphogenic pigment system, a blue-light receptor, is thought to be a flavoprotein, but little is known about the molecular basis for blue-light-induced responses. With the exception of phytochrome, which is a chromophore linked to a protein, all the known plant hormones are low-molecular-weight compounds that are active biologically at very low concentrations in the micromolar range. Past studies on the plant hormones and their active chemical analogs have chronicled the types of responses obtained when one or a combination of the classes of hormones are applied to an intact plant; to plant parts such as stems, buds, roots, and other tissues; or to individual plant cells. Often the concentration of the hormone applied is critical; higher concentrations are usually inhibitory.

The growth and development responses controlled by plant hormones and phytochrome vary. Responses indicate that many complex interactions occur among the hormones and with phytochrome. Phytochrome and the plant hormones have been shown to affect almost all aspects of development, from seed germination to flowering. Effects include growth responses to gravity (geotropism), stem elongation, bud and seed dormancy, seed germination,

cytoplasmic streaming, the orientation of cellular organelles, ripening of fruit, and the senescence of whole plants as well as plant parts such as leaves.

Much of the empirical information on the effects of phytochrome and plant hormones has led to commercial applications. For example, in the florist trade flowering plants can be produced at any season of the year by manipulating photoperiod, which acts through phytochrome. The application of auxins or ethylene precursors also induces flowering in certain species. Gibberellins are used in the brewing industry to increase the synthesis and release of hydrolytic enzymes during the malting process of barley seed. They are also used to stimulate seedless grapes to grow to a larger size. Some auxin analogs, such as 2,4 dichlorophenoxyacetic acid (2,4-D) are used as potent herbicides. Ethylene is used for ripening fruits, such as bananas, as they are shipped to market.

While much is known about the variety of effects under hormone control, the molecular mechanisms controlling hormone-mediated responses remain largely unknown. It has been difficult for researchers to determine experimentally how the active levels of hormones are regulated in the plant through biosynthesis and degradation. Also unexplained are the varying sensitivities to these hormones observed among different cell types as well as changes in sensitivity in the same cell types over time. The more recent successful efforts in research on specific hormones and the light-capturing pigment phytochrome have emphasized approaches that include: (1) an analysis of the substrates and enzymatic steps involved in the biosynthesis of the hormones, and (2) modest application of molecular biological techniques to define the effects of hormones and phytochrome on gene expression.

### Biosynthetic Pathways

Progress in working out the biosynthetic origin of the different classes of plant hormones has recently accelerated. Notable examples are the definition of the enzymatic steps in ethylene biosynthesis and the biosynthesis of the various active and inactive gibberellins.

The study of biosynthetic pathways of plant hormones and the specific enzymes involved may ultimately lead to the development of experimental tools that will help researchers understand the regulation of plant hormones at

different stages of development. Ultimately, characterization of enzymes may lead to the development of genetic probes that will assist in identifying and cloning genes that code for and regulate endogenous levels of plant hormones.

Tools, such as monoclonal antibodies that are specific for certain enzymes, are needed to identify and localize hormone biosynthesis within tissues or cells. Plant hormones are low-molecular-weight compounds that do not, by themselves, have antigenic properties. Monoclonal antibody probes for the plant hormones are now being developed, however, by covalently linking them to the surface of a carrier protein macromolecule. The carrier protein serves as the antigen to stimulate antibody production. Because the attached low-molecular-weight plant hormone has become a surface characteristic of the carrier protein, some of the antibodies produced might recognize and have affinity for free, unlinked hormone molecules.

This approach, using antibodies against plant hormones, is in its early stages. It does offer, however, a level of sensitivity for both chemical identification and quantitation that may match the physiologically active concentrations of the hormones in plant tissues. A disadvantage of this method is that tannins and other phenolic substances, often found in plant extracts, can denature proteins, including antibody proteins, and might obfuscate the sensitivity of the analysis.

Chemical Analysis Sensitive chemical analyses are greatly aiding studies in plant hormone biosynthesis. High-resolution analytical instruments are now available for the chemical identification and quantification of hormones in the plant. This analytical capability is based on the use of high-performance liquid chromatography (HPLC) followed by gas chromatography, coupled with mass spectrometry (GCMS) or nuclear magnetic resonance or both. These methods provide accurate separation, identification, and quantitation of the minute amounts of hormones present in plant tissues. The instrumentation is costly and its operation and maintenance demand special analytical skills. The accuracy and sensitivity provided by these methods, however, are often required. In addition, the biosynthetic origin and metabolic fate of these hormones in plants are being studied using radioactive and atomic mass labeling techniques.

**Genetic Variants** Single gene mutants have also been important in studies on the biosynthetic pathways of hormones in plants. For example, dwarf mutants of corn have been successfully used to study gibberellin biosynthesis. Several derivatives of the basic gibberellin chemical structure are synthesized in plants. For example, only one gibberellin, gibberellin A<sub>1</sub>, is active in controlling shoot growth in corn. Other gibberellins in the plant are important intermediates in its biosynthesis. Dwarf mutants of corn are unable to synthesize gibberellin A<sub>1</sub>; they are unable to carry out one or more of the steps in the interconversion of one gibberellin to another. Use of these mutants has been a critical tool in defining the sequences of conversions of the many gibberellins to the single active product, gibberellin A<sub>1</sub>.

In addition, mutants will be important experimental models for understanding the regulation of hormone levels during appropriate stages of development. For example, in viviparous mutants of corn, the maturing seed does not become dormant but instead continues to grow and germinate while still on the ear of corn. The dormancy of normal seed is associated with a relatively high concentration of abscisic acid. Research indicates that insufficient levels of abscisic acid are present during the maturation of the viviparous seed to impose dormancy.

### Gene Expression

Scientists have searched for specific receptor molecules that recognize and interact with a hormone or phytochrome in studies of their mechanisms of action. Radioisotopically labeled hormone molecules with high specific activity are used in attempting to locate and identify the receptor sites that bind the hormone. Thus far, this method has not succeeded in plants as it has in the case of identification of the receptor sites of steroid hormones in animals. Thus far, scientists have not succeeded in identifying a specific binding site with characteristics that correlate exactly with the physiological response induced by the plant hormone.

An alternative approach for studying the molecular mechanisms involved in hormone-related responses is to study enzymes and other gene products that appear in response to hormone application. The effect of

gibberellins on the synthesis of the starch-hydrolyzing enzyme alpha-amylase in the aleurone cells of germinating cereal grains is a classic example. Gibberellin regulates the expression of these hydrolytic genes in aleurone cells, as demonstrated by the increased levels of alpha-amylase messenger RNAs in response to the gibberellin. Similarly, the level of mRNAs coding for seed storage proteins in developing seeds has been shown to be regulated by abscisic acid.

While it is difficult to determine whether the regulation of gene expression is a primary or secondary response to the specific hormone, it should be possible to locate and clone the genes and determine how the hormone triggers the regulatory sequence.

**Photomorphogenesis** Light serves an important regulatory role in plant growth and development in addition to providing the energy source for photosynthesis. Photomorphogenesis, the light-regulated developmental changes of a plant, is primarily under the control of a pigment called phytochrome. Phytochrome regulates such diverse effects as internode elongation, leaf unfurling, flowering, seed germination, and chloroplast movement.

This high-molecular-weight pigment consists of a tetrapyrrole chromophore attached to a specific protein. Phytochrome exists in two molecular configurations that are reversibly interconverted by light. One configuration,  $P_{fr}$ , is the active form, while the other,  $P_r$ , is inactive. Red light converts  $P_r$  to  $P_{fr}$ ; far red light converts  $P_{fr}$  to  $P_r$ . Thus, changes in light quality (the amount of red versus far red light) serve as a reversible biological switch in plant development.

Research on phytochrome has focused on the chemical and physical characteristics of phytochrome and on its location in the cell as well as changes in gene expression regulated by phytochrome. Specific genes regulated by phytochrome have now been cloned. Several of these are for proteins involved in photosynthesis, such as the small subunit of Rubisco, and the chlorophyll a/b proteins. These phytochrome-regulated genes are useful in the study of transcriptional regulation of the individual genes and can be used to study the gene regulatory sequences that respond to phytochrome.

Chemical approaches that further explore the molecular structure of the phytochrome protein, immunological

approaches to localize phytochrome within the cell, and study of the regulation of gene expression by phytochrome are contributing to an understanding of photomorphogenesis and the relationships that exist between phytochrome and the phenomena it regulates. The recent observation that phytochrome controls the transcription of its own gene will have a profound effect on our understanding of photomorphogenesis.

### Cell Culture and Plant Regeneration

Two classes of plant hormones, the auxins and the cytokinins, must be added to culture media to support plant cell proliferation in vitro. While it is relatively easy to fulfill the requirements for meristematic plant cells to continue unorganized cell proliferation in tissue culture, it is far more difficult to obtain organized growth and regeneration of plants. Only certain genotypes of a species will readily regenerate from tissue culture. The factors necessary for regeneration of intact plants from tissue culture remain largely unknown. It appears, however, that plant hormones are involved, because the relative concentrations of auxin and cytokinin added to media can promote or inhibit regeneration.

The ability to regenerate plants from cell cultures at will is important to progress with gene transfer in plants. In a useful gene transfer system, DNA is introduced into a cell of the species of interest, and that cell is regenerated into a functioning plant that has been altered only by the introduced DNA.

Plant organ and tissue culture is a well-established technology that originated in the early part of the twentieth century. In certain horticultural species, use of tissue culture is a small but important industry. Progress in manipulating cultures of some major food crops, including the cereals and legumes, to achieve plant regeneration has been much slower than with other crops such as potato, tomato, and tobacco. This major deficiency in the fundamental knowledge of plant development will become an even greater constraint to research in the future unless it is closed by a major commitment to the study of plant regeneration from tissue culture. Understanding the role of plant hormones in organogenesis and growth is an important aspect of this research.

### Research Status

New and sophisticated techniques, including instrumentation for high-resolution chemical analyses, monoclonal antibodies, and methods for identifying, cloning, and sequencing genes are rapidly advancing the understanding of the regulation of plant growth and development. There is a wealth of descriptive information on the roles of plant hormones and the photomorphogenic pigment phytochrome as regulators in coordinating the development of form and function in plants. Increasing evidence points to phytochrome and plant hormones as major factors in gene expression. As the molecular understanding of gene expression in plants increases, so will the opportunities for identifying the mechanisms of action that plant hormones and phytochrome use to regulate gene expression. Alternatively, regulatory sequences of genes that respond to a plant hormone or phytochrome can be used as powerful tools in genetic engineering.

The original discovery of phytochrome and much of the outstanding early basic research conducted on this photomorphogenic pigment was accomplished by ARS scientists. The ARS should strive to reestablish its leadership role in basic research on plant growth and development. The focus of future research efforts within the ARS should include:

- Biosynthesis and degradation of plant hormones and phytochrome, with an emphasis on the regulation of genes coding for enzymes that synthesize or inactivate these substances;
- A molecular understanding of the role of phytochrome and plant hormones in regulating gene expression, particularly on their effects on the regulatory sequences of genes; and
- The role of regulatory substances in major yield-controlling processes such as flowering, fertilization, germination, and senescence.

### Physicochemical Stress

Physicochemical stresses such as drought, cold, heat, salt, and toxic ions cause extensive crop losses in the United States and throughout the world. These stresses are the main factors limiting expansion of food, feed,

and fiber production. They are the basis for unrealized production potential. This is indicated by the fact that the average yield for eight major U.S. crops including corn, wheat, soybeans, sorghum, oats, barley, potatoes, and sugar beets is estimated to be only some 20 percent of the record yield for the same crops. Of the unrealized 80 percent of the potential yield, physicochemical stress accounts for about 70 percent with the remaining 10 percent attributable to insects and diseases.<sup>1</sup>

The effects of physicochemical stress may be dramatic, killing or severely injuring whole crops. Factors causing such dramatic effects include extremes of temperatures and severe drought, such as occurred in the midwestern United States in 1983 when the average bushel per acre yield for corn in Illinois dropped 40 percent compared to the average yield for the state in the previous year. Less apparent are stress conditions that cause no visible injury but still retard plant growth and reduce crop yield. Factors causing these more subtle effects are limited water supply, unfavorable temperatures, saline soils, and the presence in the soil of toxic ions such as aluminum. Increasing the tolerance of major crops to physicochemical stress could produce enormous benefits by increasing or stabilizing productivity with little additional cost to the farmer.

The major obstacle to increasing the tolerance of crop plants to physical and chemical stresses is the lack of fundamental knowledge of the basic mechanisms of stress injury and stress tolerance. Past breeding programs for increased stress tolerance have used a trial-and-error approach based on the ability of a genotype to survive a particular physicochemical stress. Determining only survival, however, gives little indication of the specific stress effects that contribute to the dramatic drop in productivity potential noted previously. A dearth of knowledge of specific mechanisms that confer increased stress tolerance has precluded combination of compatible traits into desirable stress-resistant genotypes. In addition, conventional breeding methods allow gene transfer between closely related plants only, which restricts the available gene pool. Recent advances in plant molecular genetics, however, have opened the

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<sup>1</sup>J. S. Boyer, 1980. Plant productivity and environment. *Science* 281:313-348.



possibility of transferring specific traits between different, genetically incompatible species. But progress in conventional breeding and genetic engineering approaches will remain extremely limited until researchers have a better understanding of the effects of specific stress factors on plant growth and the effects of the genetic traits that mitigate the impact of stress factors.

### Plant Responses to Stress Factors

The effects of drought, salinity, heat, and cold on plants are usually multiple and often interrelated. Studies of the responses of plants to physicochemical stresses show that many biophysical and biochemical steps in metabolism can be affected. From this complex of multiple responses, it is often difficult to identify the primary site of damage. Thus, many response factors must be studied to determine the threshold of damage for different responses and to understand the relationships that might exist between the primary effect and the cascade of processes that are, in turn, affected.

Drought Water stress reduces or arrests plant growth because of a variety of effects. The carbon fixation steps of photosynthesis stop because carbon dioxide exchange into the leaf is blocked as the stomatal pores close to halt further water loss from the plant via transpiration. Heat energy from solar radiation is no longer effectively dissipated through the evaporation of water, and leaf temperatures may rise to damaging levels. Solar energy, absorbed by photosynthetic pigments, is no longer channeled to carbon fixation, and the photosynthetic system can be inactivated as energy is diverted to harmful reactions. Without sufficient turgor pressure the cells at the growing tips of plants cannot expand and elongate. Growth is also arrested because the products of photosynthesis needed for cell wall and protein biosynthesis are in reduced supply. Plant growth substances involved in regulating the opening and closing of stomates are also likely mediators of plant growth changes brought about by water stress.

The number of possible effects in the cascade of responses to drought will depend on the severity and duration of the water stress. Some responses, such as stomatal closure, are quickly reversed when water is

added. The kinds and degree of damage and the rates of repair or replacement are unknown. Also, drought may trigger long-term developmental changes in morphology and growth pattern that limit flowering, pollination, and seed development, which can greatly reduce crop yield.

**Salinity** The osmotic properties of high concentrations of salt ions in soil water produce the same effects as those resulting from drought. In addition to creating this state of so-called physiological drought, the excess of salt ions can cause ionic imbalances across plant membranes and in the cytoplasm that lead to impairment of metabolism.

**Low Temperature** Chilling temperatures lower the rate of enzyme activity and retard plant metabolism, including the processes of photosynthesis. Low temperature can also cause phase transitions that alter the molecular configuration of lipid components in membranes. This can result in leakage of ions and other solutes and impairment of water uptake. Such phase transitions may adversely affect the integrity and function of other important cell membranes such as the vacuolar membrane, or tonoplast, and mitochondrial and chloroplast membranes. Freezing temperatures just below 0°C disrupt membranes, especially the external plasma membrane. The formation of ice crystals causes mechanical injury to cells and produces severe water stress and excessive solute concentration.

**High Temperature** As temperatures in a plant are raised, water loss increases, thereby causing and exacerbating the effects of water stress during drought. Excess heat energy can cause metabolic imbalance by denaturing enzymes. Photosynthesis and other key processes in chloroplasts are reduced partly through loss of the integrity of chloroplast membranes. For example, increased temperature can result in increased fluidity of chloroplast membrane lipids. This may be responsible for decreases in the activity of the photosynthetic photosystems organized on those membranes within the chloroplast.

#### **Stress-Tolerance Mechanisms**

Through genetic change and natural selection, many plants have been able to adapt their physiology and

morphology to tolerate climatic and environmental extremes. Mangrove trees grow in sea water, and certain other wild species can tolerate highly saline soils. Cacti and other desert plants survive wide temperature shifts between night and day as well as periods of prolonged drought. Some plants have adapted to the frequent freezing and thawing conditions of the tundra. Others manage to tolerate otherwise toxic levels of ions found in mine spoils and in serpentine or acid soils. Many plants survive and grow in poor soils with limited nutrients. Certain plants also exhibit tolerance to atmospheric pollutants such as ozone and sulfur dioxide.

Most of the plants that have adapted to survive in truly extreme conditions are wild and not considered to be important to U.S. agriculture. Such wild plants, native to contrasting environments that are extreme in exhibiting one or a combination of stress factors, however, can provide invaluable experimental material for the identification of stress-tolerant mechanisms and genetic manipulation.

A comparison of physiological responses to stress in both stress-sensitive and stress-tolerant species is a valuable experimental approach. For example, tolerance of water stress by the photosynthetic system may in part depend on the ability of the plant to minimize the accumulation of excess excitation energy during periods of high irradiance. Plant-water relationships might also affect repair processes that may be operating both during and following exposure of the leaves to high irradiance levels. The plant's ability to maintain an adequate rate of repair, even during periods of low water potentials, may be an important stress-tolerance mechanism.

Tolerance to salinity stress may depend on the ability to accommodate osmotic changes by concentrating ions and other solutes in leaves, roots, and specialized cells. Small molecules such as proline, glycine-betaine, and polyols accumulate in some species when they are subjected to water or salinity stress. In addition to their role in osmotic adjustment, these small molecules are possible factors in stabilizing supramolecular complexes in the cytoplasm during water, salinity, and temperature stress.

#### Research Status

Research programs on physicochemical stress must be considered long range. Because the potential impact on

agriculture is enormous, this research should receive high priority within the APS.

Attention must be focused toward research approaches that characterize basic mechanisms used by plants in responding and adapting to the stresses of drought, excessive solar radiation, low and high temperatures, salinity, and toxic ions. Research approaches should also be designed to determine interactive relationships of the effects of major stress factors.

The work must be conducted at varying levels of organizational complexity to yield an understanding of the function at the whole-plant level. Research must include major plant processes such as photosynthesis, nitrogen metabolism, protein synthesis, and the transport of water, ions, and other solutes that are either excluded from or concentrated in intracellular compartments such as the vacuole and other organelles.

Comparative studies on plants that exhibit marked differences in their tolerance to a given stress factor provide a powerful approach toward uncovering the basic mechanisms of tolerance to physicochemical stresses. It is important, therefore, that the investigator be free to choose experimental plants best suited for the problem under investigation.

The three major areas of research that should be emphasized in ARS programs on physical and chemical stress are: (1) the primary sites of damage to the plant caused by a specific stress factor; (2) the mechanisms--morphological, physiological, biophysical, and biochemical--employed by stress-resistant plants to avoid and tolerate stress; and (3) the genetic bases of these tolerance mechanisms.

More specifically, the ARS must intensify research efforts in the following areas:

- Mechanisms of water and solute transport, especially into and within the roots, and the design of innovative approaches for detecting and measuring changes in the metabolism and membrane permeability of roots;
- The role of small molecules such as proline, glycine-betaine, and polyols, not only in osmotic adjustment but also in stabilizing molecular complexes during stress-induced dehydration;
- The role of excessive light as a destructive agent when photosynthesis is limited under conditions of water, salinity, and temperature stress;

- Identification of genes and gene products associated with stress tolerance in stress-adapted genotypes and species; and
- Aspects of membrane properties such as changes in the biosynthesis of major membrane constituents; temperature-related changes in lipid fluidity and membrane protein stability that affect the functional integrity of the chloroplast, mitochondrial, vacuolar, and plasma membranes; and related aspects including dehydration-induced phase transitions, freeze-induced electrical perturbations, and changes in thermomechanical properties.

## 5

# Plant Diseases and Insect Pests

The damage to plants caused by competition from weeds and by other pests including viruses, bacteria, fungi, and insects greatly impairs their productivity and in some instances can totally destroy a crop. Today, dependable crop yields are obtained by using disease-resistant varieties, biological control practices, and by applying pesticides to control plant diseases, insects, weeds, and other pests. In 1983, \$1.3 billion was spent on pesticides--excluding herbicides--to protect and limit the damage to crops from plant diseases, nematodes, and insects. The potential crop losses in the absence of pesticide use greatly exceeds that value.

For about 100 years, breeding for disease resistance has been an important component of agricultural productivity worldwide. But the successes achieved by plant breeding are largely empirical and can be ephemeral. That is, because of a lack of basic information about the function of genes for resistance, studies are often random rather than specifically targeted explorations. In addition, any results can be short-lived because of the changing nature of pathogens and other pests as new genetic information is introduced into complex agro-ecological systems.

An excellent example of the effect of genetic change is the sterile pollen trait bred into most major corn varieties to aid in the production of hybrid seed. Plants containing Texas (T) cytoplasm transfer this male sterile trait via the cytoplasm; it is associated with a particular type of mitochondrion. Unknown to breeders, these mitochondria also carried vulnerability to a toxin produced by the pathogenic fungus Helminthosporium

maydis. The result was the corn leaf blight epidemic in North America in the summer of 1970.

The methods used in the discovery of pesticide chemicals also have largely been empirical. With little or no prior information on mode of action, chemicals are tested to select those that kill the target insect, fungus, or weed but do not harm the crop plant or the environment.

Empirical approaches have produced enormous successes in controlling some pests, particularly weeds, fungal diseases, and insects, but the struggle is continuous, since genetic changes in these pests can often restore their virulence over a resistant plant variety or render the pest resistant to a pesticide. What is missing from this apparently endless cycle of susceptibility and resistance is a clear understanding of both the organisms and the plants they attack. As knowledge of pests--their genetics, biochemistry, and physiology, their hosts and the interactions between them--increases, better-directed and more effective pest control measures will be devised.

This chapter identifies several research approaches to a better understanding of the fundamental biological mechanisms that might be exploited to control plant pathogens and insects. Molecular biology offers new techniques for isolating and studying the action of genes. The existence of susceptible and resistant host plants and virulent and avirulent pathogens can be exploited to identify and isolate the genes that control the interactions between host and pathogen. Studies of the fine structure of these genes can lead to clues about the biochemical interactions that occur between the two organisms and to the regulation of these genes in the pathogen and in the tissues of the plant. It should be possible in the future to improve the methods and opportunities for the transfer of desirable traits for resistance into crop plants and, conversely, to create pathogens that will be virulent against selected weeds or arthropod pests. An increased understanding of insect neurobiology and the chemistry and action of modulating substances, such as the endocrine hormones that regulate metamorphosis, diapause, and reproduction, will open new avenues for controlling insect pests by disrupting their physiology and behavior at critical stages in the life cycle.

### Molecular Bases of Plant-Pathogen Interactions

The existence of susceptible and resistant cultivars implies specificity in plant diseases. One explanation for this high specificity is a "recognition" mechanism between pathogen and host. Understanding the molecular bases that determine this specificity in recognition or in the pathogen's ability to alter the host's metabolism should yield new, definitive, and more efficient ways to prevent attacks on crop plants or to mitigate disease symptoms.

Based on our current, limited understanding of the types of interactions that occur between host plants and pathogens, the mechanisms involved are varied and complex. Theoretically, a minimum of two criteria are involved. The first is recognition. There may be preformed molecules in both host and parasite that can interact. Second, there must be metabolic changes in the host or pathogen or both that are triggered by the initial interaction step. Genetic mutations in either host or pathogen can change the specificity of molecular interactions or their ability to trigger metabolic change.

The following presents discussions on research directed toward possible mechanisms involved in recognition between host and pathogen and the metabolic changes that cause disease symptoms.

### Molecular Determinants of Resistance and Susceptibility

It is widely held that some forms of resistance to fungal and bacterial pathogens are the result of a host plant's ability to synthesize chemicals that inhibit the growth and development of the pathogen. During infection by a pathogen, metabolic pathways in the plant are activated, leading to the detectable biosynthesis of the inhibitors. A major class of inhibitors, called phytoalexins, are primarily low-molecular-weight, secondary plant metabolites that possess wide-ranging activity against fungi and, to a lesser extent, bacteria. In the last two decades, more than 100 phytoalexins have been identified. The induction of the biosynthesis of phytoalexins, however, does not follow the specificity that most pathogens have for a specific cultivar. For example, phytoalexin synthesis can be induced by abiotic agents, such as wounding or other stress conditions, in both resistant and susceptible plants. Phytoalexins can



be toxic to both virulent and avirulent pathogens. It appears that phytoalexin synthesis might be a general, nonspecific type of active resistance.

An alternate approach, the study of susceptibility, has revealed mechanisms that show a high degree of specificity. Many pathogens possess specific agents for virulence, such as toxins or enzymes, that determine the course of events in susceptible plants. In the last five years, six host-specific or host-selective toxins have been chemically characterized. These toxins affect only susceptible cultivars and are produced only by specific pathogens that can attack these same susceptible cultivars. One well-studied example is the toxin produced by the fungus Helminthosporium maydis, mentioned earlier. The H. maydis toxin disrupts energy generation in susceptible mitochondria that characterize the T cytoplasm of corn. Normal mitochondria are resistant and are unaffected by the toxin because they apparently lack a receptor site for it.

Genetic specificity also exists for resistance and susceptibility to plant viruses, but there is no information on how such genes act. With respect to plant viruses the term resistance is used rather loosely. Quite often only the appearance of disease symptoms is considered. Thus, a plant that supports virus replication but shows no symptoms is considered to be resistant because it superficially appears to be so. More correctly, that plant should be called tolerant.

Recent observations suggest that one type of resistance may involve the ability of viruses to spread from cell to cell in their hosts. The continuum of responses ranges from rapid and complete invasion of the whole plant by the virus to slow invasion to circumstances where the virus is unable to spread from an infected cell, even though it might replicate well there. Accumulating evidence indicates that viruses induce the synthesis of proteins that are necessary for the movement of viruses from cell to cell. The host, however, depending on its genotype, can in some way interfere with this protein. Although the process is poorly understood, it may be, in part, the basis of resistance of plants to viruses.

In a sense, viruses might be thought of as packages of genes; they are composed primarily of RNA or DNA, and they can replicate only in a favorable host cell environment. Studies of the interactions between viral

RNA or DNA and genes in the host cell can lead not only to an understanding of how viruses function but also to the development of viruses as gene-carrying vectors for genetic engineering.

An improved understanding of the basic concepts controlling resistance and susceptibility will result from research based on interrelated approaches to the analysis of the genetics of these traits, the gene products, the structure of the genes, and the methods that will permit their transfer between organisms.

Genetics Continued breeding studies and genetic analysis of resistance traits in host plants and virulence traits in pathogens provide the experimental systems needed to isolate and determine the properties of recognition molecules involved in susceptibility or active resistance, such as phytoalexin biosynthesis.

Single-gene changes that confer resistance against a pathogen exist and are used in crop breeding to develop improved cultivars. In other cases multiple genes appear to be involved in resistance, and complicate crop breeding. The growing collection of data on the genetics of host plants and particularly of pathogens needs to be strengthened. Such data are essential for identification of the genes that control the specificity of receptor molecules, which determine resistance or susceptibility to bacteria, fungi, or viruses. Genetic analysis of some important fungal pathogens, however, will be difficult because sexual reproduction does not occur, and the modes of genetic reassortment and inheritance are unknown.

Many genetic approaches are now being initiated. For example, single-pathogen genes responsible for disease reactions in two bacterial leaf-spot diseases, soybean blight and bacterial spot of tomato, are being isolated and cloned. These techniques have potential for wide application.

Gene Products The end product of most genes is a protein. There is little direct evidence for the role of any specific proteins in controlling interactions between a host and a pathogen. Many potential candidates, however, can be hypothesized. By analogy with animal systems, surface molecules, such as membrane glycoproteins, may interact with low-molecular-weight messenger molecules, such as small carbohydrates released from cell walls. Cell wall extracts from both hosts and pathogens have

been shown to elicit some resistance responses. Both the hydrolytic enzymes that release carbohydrate fragments from cell walls and the enzymes involved in the biosynthesis of toxins or phytoalexins are gene products that may be selected for study.

Additional basic information is needed about the cellular interactions between host and pathogen during the onset of resistance reactions. For example, the precise mechanisms employed by phytoalexins to exert their effects on pathogens are unknown and need to be actively studied. Metabolic pathways for the biosynthesis of phytoalexins must be clarified, and other compounds associated with resistance need to be identified. The regulation and coordinated synthesis of the enzymes involved in these pathways must be detailed.

In addition, the phenomenon of acquired resistance in plants needs further study. Resistance can be localized or can occur throughout the plant. Systemic resistance, however, may be of more practical value. This phenomenon can appear after a host plant is inoculated with an avirulent strain of the bacterial, fungal, or viral pathogen. This exposure somehow induces resistance properties so that when the plant is subsequently challenged by one or more pathogenic strains, it will resist infection or exhibit only mild disease symptoms.

Acquired resistance is most actively being studied using *Pseudomonas solanacearum*, some strains of which cause wilt and stem rot in tobacco, ginger, potato, tomato, and banana. Other avirulent strains only induce resistance. The experimental approach is to find mutants of the avirulent strains that fail to induce the acquired resistance. A comparison of the gene libraries of the active with the inactive mutants could lead to the identification of the genes and gene products responsible for triggering the acquired resistance.

Gene Structure Once the genes and gene products are identified, it is feasible to alter their activity by changing the structure of the gene itself. The tools of molecular genetics can be used to study both the structure and activity of pathogen genes for virulence and avirulence and host genes for resistance and susceptibility. Some progress has been made recently with bacterial pathogens, particularly in characterizing some virulence factors such as pectolytic enzymes. Much of the basic information on the molecular biology of fungal pathogens, however, is yet to be acquired.

The functions of proteins coded for by viral genomes must also be established to aid in the understanding of their possible roles in replication and pathogenesis.

It may now be possible to isolate genes for specific types of resistance, such as that characterized by so-called hypersensitive lesions. For example, certain plant species and cultivars respond to infection by a pathogen by rapidly undergoing cell necrosis at the site of infection. The hypersensitive lesion can effectively stop the spread of a virus or confine the bacterial or fungal pathogen. In the latter two cases, the pathogen then dies.

This response is controlled in most cases by a single, dominant gene in the host plant. One approach to study of the mechanism controlling development of the hypersensitive lesion would be to first isolate messenger RNA from infected plants--those induced to give a hypersensitive response and those with a suppressed hypersensitive response. The mRNA from the suppressed plants could be used to prepare complementary DNA. This complementary DNA should recognize and hybridize with all the mRNAs from induced plants, except for those involved in the hypersensitive response. In principle, the remaining free mRNAs could then be used to probe a gene library of the hypersensitive plant for the gene that they can hybridize with. This gene should be the one responsible for inducing the hypersensitive lesion.

Gene Transfer The ultimate goal of research discussed in this section on genetics, gene products, and gene structure is the routine transfer and expression of genes for resistance in agriculturally useful plants. As noted in the earlier chapter on genetic engineering, some bacterial and viral pathogens may be developed as suitable carriers for the transfer of genes into host plants. Current and prospective vectors take advantage of naturally occurring, intimate associations between microorganisms and plants, both pathogenic and beneficial. An appreciable effort is needed to identify and obtain suitable vectors in addition to the one successful vector, the Agrobacterium Ti plasmid that can be used in some dicotyledonous plants.

The techniques necessary to manipulate vectors are available and will likely be refined and improved within the next few years. It is, unfortunately, the lack of knowledge of the basic biology of plants and of the function, transfer, and expression of genes that restricts progress in this area.

### Molecular Basis of Cellular Damage in Susceptible Hosts

Although it may appear that research on cellular damage and disease symptoms is a subset of the research discussed previously on resistance and susceptibility, its intent is distinct, but of equal major importance. Research emphasis in this area will yield insights into the biochemical mechanisms that result in cellular damage, or disease, following successful pathogenic invasion. As yet there is no clear explanation of how major symptoms, such as the yellowing and loss of chlorophyll in chlorosis or the tumors, galls, and morphological changes caused by cellular growth distortion, are induced once a virulent pathogen becomes established in a tissue. It may be possible to ameliorate symptoms or prevent crop damage directly by treatment, if the biochemical details are known. The little-understood phenomenon of natural tolerance to disease is evidence that such treatment should be possible. Indeed, the study of natural tolerance may be a valuable guide for developing disease protection traits for crop improvement.

Easily observable disease symptoms, such as chlorosis, necrosis, and cellular growth distortions, can have a number of diverse causes. Therefore, it is not possible to make progress on such generalized disease symptoms without some indication of the kinds of pathogens involved. Some research approaches hold promise for establishing general scientific principles of host-pathogen interactions.

Mode of Action of Toxins Research in the last decade on purification and structural characterization has led to an acceptance of the concept that toxins are the potent chemical agents of virulence in many important diseases caused by bacteria and fungi. Only a small number of toxins have been chemically identified. Even fewer have a postulated target or receptor site in the host cell, as was described earlier for Helminthosporium maydis. But even in these few cases, it is not known how interference with the target site leads to cell damage. Much additional research is needed on toxins--on their genetics, such as chromosomal versus plasmid inheritance; on their chemical structure; on the pathways of biosynthesis in pathogens; and on their biochemical effects and role in pathogenesis.

Nucleic Acid Interactions It is clear that the mere replication of a virus or viroid within a plant does not determine whether that plant will be diseased. There are many examples of strains that produce a great deal of virus, but with very little damage to the host. On the other hand, some of the most serious plant diseases are caused by viruses that replicate very sparingly.

Viruses, with their small genomes, have too little genetic information to code for the variety of proteins necessary to account for the almost infinite number of symptom types. Thus, it seems likely that interactions between the nucleic acid of the pathogen and that of the host initiate the disease process. Viroids, which are RNA molecules that do not code for a protein product, can cause symptoms similar to those caused by viruses. This lends support to the supposition that viruses as well as viroids interact directly with the genome of the host plant.

Complete nucleic acid sequences are now available for several viroids; for satellite RNAs, which modify the symptoms of their carrier viruses; and for a few plant viruses. Complete complementary DNA clones have been made for some of these RNA agents and have been shown to be infectious. Because DNA is technically easier to modify than RNA, such DNA clones provide the opportunity to make site-specific modifications in the sequence of the nucleic acid by inserting or deleting short stretches of DNA. The effect of such changes on the agent's ability to infect and on the symptoms produced can then be determined.

Using current methods the nucleotide sequences responsible for the disease syndrome should be identified. Furthermore, these complementary DNA clones could also be used in hybridization studies to locate regions in the host genome where the host and the virus, satellite RNA, or viroid sequences interact. As knowledge of the fine structure of the host's genes increases, future studies should enable researchers to determine the specific genes and processes that are perturbed by the presence of the pathogen.

If the DNA clones themselves are not infectious, the cloned viral or viroid DNA can be transcribed back to RNA using any of several in vitro systems. Thus, site-specific modifications made in the DNA clone can be transcribed into the RNA to test the effect of such changes on infectivity and disease symptoms. In this manner, critical regions of the genome could be identified,

which would aid in the understanding of their functions and possibly the functions of viral-coded proteins.

**Bacterial Interactions** Bacteria that cause diseases in plants cause symptoms, at least in part, by the production of various metabolites. Relatively few of these substances have been identified. The metabolites include, but are not limited to, toxins, polysaccharides, pectic enzymes, and plant hormones. All the bacterial toxins identified to date appear to be general toxins affecting a wide spectrum of plants. Many of these plants are not considered to be host species for the bacterial pathogen producing the toxin.

Other bacterial metabolites appear to have specific effects on host plant species. Bacterial polysaccharides, which are associated with wilting of plants, can be released in amounts great enough to clog up transport between plant cells, and may act by disrupting plant cell membrane functions. Soft rots, for example, are the result of bacterial enzymes that degrade the cementing pectin layer between plant cells. The production of plant hormones by bacteria disrupts the endogenous hormone balance in the host plant and can be part of the mechanism leading to crown gall tumors and other abnormal growths.

The molecular and genetic bases of the synthesis of these pathogen metabolites and the basis of the symptoms they cause in the host plant are largely unknown. There is increasing knowledge, however, about the genetics of some of the bacterial virulence factors that contribute to the severity of a disease. For example, in crown gall, which is caused by Agrobacterium, both bacterial chromosomal and plasmid genes are known to be required for pathogenicity. The molecular genetics of crown gall is the most thoroughly studied of any plant disease.

In the genetic analyses of virulence in bacteria, two different approaches are currently being used. One is the introduction of transposons into virulent strains of bacteria to create avirulent mutants. The transposon is used as a probe to locate and isolate the turned-off virulence gene. DNA clones of virulence genes can be used for an analysis of gene products. The second approach is molecular and genetic analyses of known or suspected determinants of pathogenicity, such as cell surface components, hormones, toxins, and extracellular enzymes. Both approaches hold promise for the elucidation

tion of the biochemical steps in pathogenesis. There is an essential need to have basic knowledge about the structure, function, and regulation of virulence factors in the pathogen to provide a basis for directed plant breeding and to design effective inducers of plant resistance.

#### Research Status

It is important to recognize that considerable expertise and training in molecular biology are necessary for many of the research approaches discussed in this section of the report. Progress is facilitated by individuals working together in groups. Interactions with researchers in other laboratories are important sources of intellectual stimulation as well as sources of technical expertise.

The tools of genetics and molecular biology offer some new methods for understanding the highly specific interactions between host and pathogen. Studies of the molecular aspects of plant pathology must receive high priority and emphasis within the ARS research programs on plant diseases.

Currently the ARS research centers are undertaking relatively little basic work in molecular plant pathology. The ARS does have a few strong research programs in virology and in viroids, but very little work at the molecular level is being conducted with bacteria or fungi. A single laboratory, at Beltsville, is studying plant mycoplasmas.

To strengthen programs in the molecular basis of plant diseases, research investigations should emphasize:

- The molecular bases of the factors that determine whether a host-pathogen pair will result in a resistant or a susceptible interaction.
- The basic concepts of the interaction between the host and the invading pathogen that result in disease. This should lead to novel methods of preventing damage from disease, including natural plant tolerance.
- The transfer of resistance traits to normally susceptible plants through the development and subsequent exploitation of vector systems that allow for gene transfer between plant species.

It is significant to note that very few laboratories in the world have undertaken studies to understand



the molecular basis of plant diseases from gene identification to disease symptoms.

#### **Modification of Microorganisms for Biological Control and Organic Pesticide Disposal**

The reliance on chemical-based pesticides, the increasing occurrence of pesticide resistance, particularly in insect pests, and the potential of such agricultural chemicals for polluting the environment are of increasing concern. The search for ways to address these concerns has led to greater emphasis on biological control. Biological control methods involve the use of one organism to mitigate the undesirable effects of another. Two complementary approaches are (1) the identification, biological characterization, and genetic engineering of crop-enhancing microorganisms, especially those that can be applied to seeds or roots to promote improved growth or yield; and (2) the development of genetically engineered microorganisms to remove organic pesticide residues, such as herbicides.

#### **Microbial Agents for Biological Control**

Little commercialization of microorganisms for biological control has been done. Yet there is great potential for research and development in this area. Knowledge of the basic biology of viruses, bacteria, fungi, nematodes, insects, and weeds is essential for identifying and developing naturally occurring antagonists as biological control agents. This includes knowledge of the growth and metabolism of the organisms obtained from both laboratory and field studies. Fundamental knowledge of the biological basis of the control mechanism and the ecology of both organisms involved is needed to successfully manipulate them to full advantage.

Interactions among several disciplines will be necessary. Soil physicists, meteorologists, computer modeling experts, and analytical chemists, as well as biologists with expertise in areas such as ecology, microbiology, and genetics will be essential contributors. The potential opportunities to use pathogens and pests against terrestrial and aquatic weeds, and against pathogens of agronomic crops, forest trees, and orna-

mental plants are enormous. Biological control is not generally expected to substitute totally for chemical control, but will supplement or be integrated with it.

Control of Pathogens The proven feasibility of using biological control in this area has spurred research. The bacterium Agrobacterium radiobacter is commercially used to prevent infection of susceptible plants by a related bacterium, A. tumefaciens, which causes tumorous galls to form on many plants. The fungus, Peniophora gigantea, is used to control another fungus, Heterobasidium [=Fomes] annosum, which causes root rot of pine trees. In both these cases the control mechanism is not completely understood, but is believed to result from competition between the control microorganism and the disease-causing organism for specific binding sites on the host plant. Also, it may be that the control organism can elicit a resistance reaction in the host. In the case of A. radiobacter, an antibiotic, agrocin 84, produced by the A. radiobacter has been identified as the possible mechanism in that example of biological control.

Biological control is also illustrated by the epiphytic bacterium Pseudomonas syringae. Both pathogenic and nonpathogenic strains of this bacterium are known to synthesize ice-nucleating proteins. When these bacteria on the plant surface are killed by antibiotic treatments or displaced with mutants of P. syringae that have lost their ability to synthesize ice-nucleating proteins, the plant can tolerate chilling to  $-7^{\circ}\text{C}$  without frost damage. Mutant strains have been produced both by mutagenesis using chemicals or ultraviolet irradiation and by removing the ice-nucleation protein gene using genetic engineering techniques.

Control of Insect Pests Alternative strategies for the control of insect pests need to be developed to augment the chemical and biological approaches currently in use. Some success has been achieved with Bacillus thuringiensis, used commercially for the biological control of some insects. This bacterium, when ingested, is lethal to the caterpillar stage of many insects. The bacterium harbors a toxic crystalline structure that dissolves in the alkaline hind-gut of susceptible caterpillars, resulting in disruption of digestion and death.

The use and genetic manipulation of insect pathogenic bacteria and viruses constitute a promising but comparatively underdeveloped approach to insect control. The potential exists for genetically improving these organisms to increase their pathogenicity, either by enhancing existing pathogenic traits or by introducing desirable pathogenic characteristics.

Basic knowledge about potentially useful pathogens must be acquired. This includes identification of the pathogen and characterization of the insect host. The specificity between pathogen and host and the techniques for production and storage of candidate pathogens must also be studied. With this information the physiology, biochemistry, and genetics of the host-pathogen interaction can then be investigated. More specific areas of study include the molecular basis of processes such as recognition, virulence, and toxicity and the mechanisms regulating gene function during these interactions.

Progress in this line of research is apparent from the work of many laboratories worldwide. Candidate microorganisms identified by this research include baculoviruses and Bacillus thuringiensis. With recent developments in insect cell culture, some of the fundamental processes detailed here, in principle, can be directly probed in vitro with any of these microorganisms.

Control of Nematodes Control of plant parasitic nematodes has been largely accomplished through the use of chemical nematocides, many of which have now been shown to be harmful to the environment and have been withdrawn from use. Biological control measures using resistant plant varieties and trap crops have been effective in some cases. A trap crop can stimulate the hatching of nematode eggs but does not support nematode growth, thus reducing nematode populations to harmless levels.

More information is needed on the basic biology of nematodes to provide directed approaches to their control, using less toxic, target-specific substances. This might include the use of the hatching stimulants that are apparently produced by plants and trigger nematode eggs to hatch. The growing nematodes then perish in the absence of a suitable host plant. Studies of nematode pheromones and hormones could lead to methods for controlling reproduction or development.

**Plant Health Microorganisms** In recent years some information has been gathered on soil microorganisms, specifically, certain bacteria, that can improve plant vigor and contribute to increased yields. The mechanisms by which such bacteria exert these effects are essentially unknown, nor are their relationships to pathogens or other microorganisms in the environment well understood. Indeed, candidate organisms suited for particular crops remain to be identified and characterized. Such bacteria contribute a desirable and perhaps essential microflora for optimal plant growth. While a range of microflora is known to be essential for human health, virtually nothing on a comparable basis is known for plants.

Several mechanisms have been suggested that describe the effects of soil microorganisms on plant health. Beneficial microbes may produce antibiotics that inhibit the growth of pathogens, or they may be involved in the acquired resistance phenomenon. Recent evidence suggests that some plant growth-promoting bacteria produce siderophores, iron-chelating molecules, that restrict the availability of this essential element to pathogens and other members of the microflora.

**Biological Degradation of Organic Pesticides** Timely and appropriate disposal of pesticide residues in water and soils is an important and attainable goal in routine agricultural production practices. The biological degradation of pesticides is theoretically feasible. For example, pseudomonads have been identified as being able to degrade the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) to innocuous compounds. Lack of knowledge of the chemistry, the fate of breakdown products, and the ecology of the organisms involved, however, is still a constraint to their use.

Both waste disposal of agricultural by-products and biomass reduction on an industrial scale are under intensive investigation. The processes are not commercially feasible as yet, however, because of low yields and organism management problems. These problems can be overcome using genetically engineered organisms, especially bacteria, that are currently more amenable to manipulation than other microorganisms.

#### **Research Status**

The ARS laboratories are among those contributing to progress in biological control of plant pathogens and

insect pests. With increased emphasis, the ARS could be at the forefront of this research. The potential return for the ARS extends beyond the control of plant pathogens and insect pests; it would involve the development of general methodologies for gene transfer, cloning, and gene expression using microbial and insect systems. Basic research on the microflora of the rhizosphere is also an area that ARS can strengthen.

There is enormous potential for the identification, development, and application of microorganisms that can degrade pesticide residues and other toxic wastes. The ARS should expand its efforts--some of which are exemplary--in these areas. It is high-risk, long-range research and requires the multidisciplinary base that is already in place in some locations.

Specifically, the ARS should focus research toward:

- Exploring and identifying microbial agents that can control plant diseases and insect pests. Further, the agency should seek conventional genetic or recombinant-DNA technologies to make these agents more effective;
- Generating more knowledge of the basic biology of plant pathogenic nematodes to develop novel, nonpesticide means of control by perturbing reproduction and development; and
- Developing unique microorganisms that will promote plant health and others that can be used to detoxify or destroy organic pesticide pollutants.

#### Molecular Basis of Pesticide Action

Pesticides are major tools in the production of food and fiber and in the maintenance of high standards of veterinary, human, and plant health. Better pesticides are needed, relative to cost effectiveness, potency, selectivity, persistence, environmental impact, and safety for domestic animals, humans, and plants. Most of the early pesticides were discovered in industrial programs involving the synthesis and screening of thousands of synthetic chemicals for safe and effective molecules. The emphasis in current discovery efforts favors research on the natural chemicals produced by plants and microorganisms, such as occurred for the pyrethroids. Equally important are investigations into the molecular basis of pesticide action.

Advances in bioregulation research provide new vistas in seeking enzyme or receptor targets for pesticide action. Increasing fundamental knowledge of the function and regulation of communication systems within living organisms focuses attention on new targets and greatly facilitates the molecular design of optimal compounds for pest control. Greater diversity is needed in the targets for future pesticides, such as insecticides, herbicides, nematocides, and fungicides to avoid or minimize the impact of pesticide resistance and toxicity against non-target species. Susceptible and tolerant species often differ only in the sensitivity of their pesticide receptor site or their facility for detoxifying the pesticide.

A clear definition of the mechanisms involved will provide the background for the next generation of improved pesticides. New pesticides, in turn, provide unique probes to explore cellular entities such as enzymes, receptors, and membranes.

The molecular basis for metabolic activation and detoxification must be defined. Using this background knowledge genetic engineering can provide opportunities for modifying receptor sites and detoxification mechanisms for improved animal and crop safety.

#### Research Status

Research on the molecular basis of pesticide action is carried out in many laboratories within industry, universities, and the ARS. Industrial labs tend to focus on the modes of action of their proprietary compounds. Universities more often use pesticides as probes for physiological and pharmacological investigations. The ARS has placed considerable emphasis on the mechanism of pesticide action. The laboratory defining a new target often reaps the benefit of finding alternative agents working at the same site or in the same way.

Research on pesticide mode of action requires the creative teamwork of biochemists, chemists, and geneticists with adequate instrumentation and the appropriate environment to stimulate communication. This multidisciplinary approach and the requisite personnel are now in place in several ARS laboratories. The ARS should increase its emphasis on the molecular basis of pesticide action, using the available expertise in microbial, plant, and insect physiology, biochemistry, and natural

products chemistry. Success in this program will serve as the basis for improving animal health and for reducing crop losses during production and storage.

More specifically, the ARS should emphasize:

- Definition of the molecular basis for metabolic activation and detoxification of pesticides;
- Study of new targets for selective pesticide action;
- Identification of new natural chemicals important in regulating pest populations;
- Investigation of the basic molecular biology of vectors for gene transfer and elucidation of gene regulation in insects; and
- Continued research on both insect genetics and on natural products chemistry.

#### Insect Neurobiology and the Regulation of Development and Reproduction

The functional responsiveness of an insect is dependent on rapid chemical communications among its own cells and between the individual and other insects. Intercellular communication is mediated primarily by the nervous system, through substances such as neurotransmitters, neurohormones, and neuromodulators as well as by the endocrine system, through hormones. The endocrine system is closely coupled to the functioning of the nervous system. Communication between individuals is achieved through volatile chemicals called pheromones. Their production and action is mediated by the nervous system.

#### Insect Neurobiology

The function of the nervous system makes it a logical focus for investigations of alternative means of insect control that could potentially have considerable selectivity. Before investigations can be initiated, however, basic information about the function of the insect nervous system must be obtained, specifically, information about nervous processes involving chemical communication. This approach is the only potentially successful avenue to the solution of applied research problems. For this reason a research emphasis in

fundamental insect neurobiology should be developed by the ARS.

Insect neurobiology is now experiencing a period of exponential growth. Despite the fact that the insect nervous system has been used for many years as a model for studying certain neurophysiological processes, basic research using modern techniques has only recently begun on the neurochemistry, neuroendocrinology, neurogenetics, and neuropharmacology of the insect nervous system.

For example, the number of identified insect peptides with neurohormonal activity is fewer than 20. Only 4 of these insect neurohormones have been purified and sequenced. These include the neurotransmitter/neuro-modulator proctolin, the two adipokinetic hormones, and cardiac accelerator peptides. Proctolin is important in the stimulation of muscle contraction and is co-released with other neurotransmitters. The adipokinetic hormones mobilize lipid for its metabolism by muscle in insect flight, and the cardiac accelerator peptides control the heartbeat of the insect. It now appears that the structures of the prothoracicotropic hormones, the primary effectors of insect metamorphosis and the first hormone of neural origin described for any animal (1917), are finally being resolved. In addition, a new brain peptide that regulates the production of pheromones has been described and promises to introduce a renaissance in pheromone research.

Study of these and of yet-undiscovered hormones will aid in an understanding of the physiology of the insect, its growth and development. Such studies will also define the mechanisms by which the central nervous system integrates and regulates these processes. This understanding may allow scientists eventually to selectively manipulate the neuroendocrine system, and thus control insects by altering their ability to fly, curtailing metamorphosis, or disrupting sexual recognition. The study of neurohormones may not provide an immediate answer to insect control. The resulting knowledge, however, will provide scientists with the sound foundation necessary to propose and pursue new directed and applied research on the neural regulation of insect growth and development.

The top scientific priority for neurobiological research on insects is the elucidation of the mechanisms by which chemical communication directs and coordinates the growth, development, homeostasis, and reproduction of



insects. The basic information still lacking includes the identification of neural regulators and an elucidation of their chemistry, synthesis, secretion, and metabolism.

Other opportunities for manipulation of insect pests include the neurohormones bursicon, diuretic hormone, and egg development neurotropic hormone. Bursicon causes the insect skeleton to harden. Inhibition of the secretion of this hormone would cause death. Manipulation of the diuretic hormone, which regulates water and salt balance, might also result in death, through ionic imbalance and dehydration. Secretion of egg development neurotropic hormone from the brain of the female mosquito is stimulated following a blood meal. The hormone indirectly causes the ovary to mature the eggs. Manipulation of this reproductive hormone would prevent the development of generations of mosquitoes.

These hormones are examples of the potential in this field. To realize this potential the hormones must be studied extensively at the chemical, molecular, and physiological level.

At this point a major research program encompassing the physiology, biochemistry, and molecular biology of these regulators can be initiated. Research should include the study of mechanisms of communication within the nervous system, between organs and organ systems, and between individuals of the same species. Studies of interorganismal communication should emphasize the neuroendocrine and neural bases of this process and relate this communication to behavioral patterns in nature.

Knowledge gained from such a fundamental research program in insect neurobiology could be used in conjunction with genetic engineering methodologies to investigate the basic molecular biology of vectors for gene transfer and to elucidate gene regulation in insects. These new technologies could also aid in mapping the insect genome, particularly the genes for regulatory peptides.

Peptides offer researchers an extremely important direct line of study; they probably are all products of single genes. An understanding of these gene products or polyprotein precursors and their posttranslational processing to a bioactive peptide is essential for the potential control of insects. (Posttranslational processing, which follows the translation of RNA, is proving to be a fundamental mechanism that determines the protein

nature of the neurosecretion from a given cell.) A disruption of the synthesis or processing of neurohormones would be lethal.

The long-term goal of this research is modification of the normal function of the insect nervous system to affect viability. A research program on insect pathogens as vectors for gene transfer would clearly be important in achieving this objective.

#### Endocrine Regulation of Metamorphosis, Diapause, and Reproduction

The postembryonic development of the insect involves a series of dramatic physiological and biochemical transformations that culminates in its emergence from a pupa as an adult form with its own unique function. It is generally accepted that these transformations and their associated metabolic processes all are directly or indirectly under endocrine control, including production of hormones by neural tissue. The full extent of the role of the endocrine system is not completely known, mainly because of a lack of knowledge of the hormones involved, the molecular basis of the developmental and reproductive processes these hormones control, and their mechanisms of action. The progress made in this field in recent years has largely been at a descriptive level. Thus, basic research is needed to identify and chemically characterize insect hormones and to define at the molecular level both their physiological function and their mechanism of action.

Although some insect hormones, such as the sesquiterpenoid juvenile hormones and the ecdysteroids, have been intensively investigated, the extent of their involvement in regulating insect development and reproduction is only now being realized. They are known to exist as structural and functional families of molecules, each acting at a specific time during the life cycle of the insect. The multiple functions of these hormones provide multiple avenues for pursuing control of the insect. Substantial stantially more research is needed, both in the above-cited areas as well as on the mechanisms of their interaction at the level of the target gland and interendocrine feedback control. Research studies must be designed to show how these hormones regulate one another's synthesis and secretion to drive development and growth.

A virtually unknown family of insect regulators that control metamorphosis, diapause, and reproduction is the peptides. Only a few have thus far been identified, and as has proved to be the case with vertebrates, there are numerous peptide hormones involved in the control of embryogenesis, postembryonic development, reproduction, and homeostasis. These peptides need to be characterized, their physiological functions defined, and mechanisms of action elucidated.

The regulation of the synthesis, secretion, and metabolism of these insect hormones, whether peptide, steroid, or other chemical structure, is another relatively unexplored research area of considerable significance and potential application to the control of insects. The secretion of these hormones has consistently been shown to be precisely regulated, frequently in response to discrete environmental cues such as photoperiod, temperature, and stress. The mechanisms by which these cues are transduced by the nervous system to elicit an endocrine response are important areas for basic research in insect neurobiology.

Knowledge of the regulation of insect development and reproduction is applicable to the manipulation of these systems for improved pest control. Some natural and synthetic chemicals, including insecticidal compounds, alter growth and development by inhibiting the biosynthesis or action of juvenile hormones or ecdysteroids and by governing the initiation and termination of diapause. Certain antibiotics and the highly insecticidal benzoyl-phenyl ureas interrupt chitin synthesis necessary for the formation of the insect cuticle or skeleton. Studies on insect genetics indicate the possibility of breeding sterile hybrids for use in pest control. Bacteria and other microorganisms producing insecticidal materials and the plant itself may also be modified by selection and genetic engineering to increase the impact of natural toxicants or feeding deterrents in host-insect pest interactions. Further development of insect cell cultures and vectors for gene transfer in insects may permit the introduction of deleterious effects into pest populations.

The benefit from research in insect neurobiology is not the potential control of insect pests alone. Although the insect is a relatively simple system structurally, it is functionally complex, much like that of vertebrates. An understanding of the insect endocrine system will lead to a further understanding of similar processes in all eukaryotic organisms.

### Research Status

The ARS is recognized worldwide for developing the sterile insect release method of control and for investigations on insect genetics and ecdysteroids. The agency also is internationally recognized for natural products research, particularly pheromone chemistry, and the application to insect development and reproduction. This type of interdisciplinary research requires a coordinated team of entomologists, physiologists, biochemists, and chemists.

There are a number of ARS laboratories currently conducting excellent research on the physiological and chemical aspects of endocrine control of insect development and reproduction. By bolstering these existing programs with the appropriate additions of scientists skilled in protein chemistry, basic biochemistry, and the study of nuclear and membrane proteins as receptors, the ARS should be able to make substantial contributions to this research area.

Although the ARS is becoming increasingly more involved in fundamental insect neurobiological research, this program is not developing in a focused manner. While most of the research skills necessary for a major program in insect neurobiology--chemistry, neurophysiology, behavior, biophysics, and physiology--are already in place within the ARS, additional expertise in neurochemistry, peptide chemistry, and biochemistry (mechanistic aspects or chemical regulation), and immunology must be added. Generally, adequate instrumentation for this research exists within the ARS. Analytical facilities are needed, however, for peptide and neurotransmitter structural identification.

Of the few laboratories worldwide engaged in insect neurobiological research, a number are emerging as centers of excellence. The comparative paucity of such centers, however, means that relatively few neurobiological systems are currently being explored. Thus, the scientific opportunities in this field are enormous. Unfortunately, the lack of basic information has created a situation wherein the most important areas of research are high risk and will require considerable effort and resources. Such high-risk research is well suited for government-supported organizations like the ARS.

To date, a multidisciplinary program in insect neurobiology does not exist. The ARS has an opportunity to establish the first program of this kind. The success of such a program greatly depends upon the centralization of

research at a single site, preferably near a university or another research institute that has a strong program in neurobiology.

ARS research should specifically focus on the following:

- Chemistry of the brain factors that control pheromone production and release, and their mechanisms of action;
- Neural regulation of the synthesis, processing, and secretion of cerebral pheromotropin peptides;
- The endocrine basis of insect reproduction, in particular, identification of the cerebral neuropeptides involved and their target glands, and identification of the mechanisms regulating these glands;
- Mechanisms that regulate the synthesis of ecdysteroids and juvenile hormones, and the biosynthetic pathways of these two hormone families; and
- Interhormonal endocrine feedback; regulation of insect growth, development, and reproduction; and the roles and molecular mechanisms of the principal developmental hormones in regulating one another's synthesis.

## 6

# The Optimal Climate for Basic Research

In addition to identifying the most promising opportunities for agricultural research, the Committee on Biosciences Research in Agriculture unanimously insisted that an optimal climate for basic research is at least as critical to productive science as the specific areas of research that are pursued. This chapter summarizes the committee's recommendations on research climate, based on 23 visits to 19 different ARS sites, and the collective experiences of the committee members. The recommendations, for the most part, are applicable to modern basic biological research, both within and outside the ARS.

### Introduction

Scientific research is most elegantly described by the unending pursuit of ideas and the pathways of experiments. It is also characterized by the flow of researchers in and out of laboratories, their personalities and influence, publications, instrumentation, the network of communications, and the overall structure and policies of the institution.

In their study Zenzen and Restivo<sup>1</sup> state:

Scientific knowledge is created out of available resources--including formal and informal modes of communication, and instrumentation. In the deepest

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<sup>1</sup>Zenzen, M., and S. Restivo. 1982. The mysterious morphology of immiscible liquids: A study of scientific practice. *Social Science Information* 21:447-473.

sense, the available resources in a given laboratory refer to the researchers' capacities for creative and critical thought, persuasion, communication, conflict and cooperation. The indeterminacy of scientific criteria, the "looseness" of laboratory research, provide room for the exercise of those capacities.

These factors, obvious and yet seemingly peripheral to the progress of science, compound to create a certain climate for research. Scientific progress is enhanced by a climate that offers the researcher and the program itself the flexibility to follow varying tracks of a problem, and that encourages immediate communication and exchange in the form of attendance at scientific meetings, sabbaticals, and participation in seminars. Now, with the quickening pace of technological innovation and the increasing importance of a multidisciplinary approach in research, climate becomes even more important as an influence that can be optimized in a number of ways.

The lag time between basic research and technological application is shrinking; the growing biotechnology industry, for example, is drawing largely from the biology of the past 10 years. Floyd E. Bloom in his summary of Frontiers in Science and Technology<sup>2</sup> states:

In such an era of rapid transformation, the structures for basic research and technological development must be dynamic and must be constantly freshened by the infusion of new and highly trained scientists and engineers, by the very best instrumentation, and by unfettered communication of fundamental knowledge.

The new biology, at its accelerated pace, brings with it the need for program and organizational changes and streamlined communications--visits to other research laboratories and the technologies that provide access to

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<sup>2</sup>Bloom, F. E., 1983. Introduction: Science, technology and the national agenda. Pp. 1-13 in Frontiers in Science and Technology, a report by the Committee on Science, Engineering, and Public Policy of the National Academy of Sciences, National Academy of Engineering, Institute of Medicine. New York/San Francisco: W. H. Freeman and Company.

discussion through conference telephone calls and data base searches. New biology scientists require not only advanced instrumentation, but more importantly, increasing numbers of postdoctoral researchers in their laboratories, providing for the exchange of fresh perspective with experience.

All research organizations are attempting changes to stimulate new means of multidisciplinary research and development. Many private corporations are developing significant internal postdoctoral research programs in the biological sciences. Among the benefits are the rapid infusion of new ideas and capabilities as well as the incorporation of flexibility that a continuum of postdoctoral employees provides. Such an approach has been and is being extensively used in universities and at the National Institutes of Health in Bethesda, Maryland, where, in fact, approximately 50 percent of the total staff are nontenured or of a postdoctoral status.

Rigid priorities, particularly long-term priorities, can no longer be set as planners may still envision. As stated previously, research priorities and directions must now be broad enough to readily take advantage of unexpected results and new strategies for resolving research problems, but also, the setting of rigid disciplinary priorities has become impractical as the face of science changes. The techniques that have evolved through an increasing knowledge of molecular and cellular genetics apply to all living things, from viruses to humans. These newer techniques influence all the biosciences; they clear the way to better communications among researchers and to collaboration among scientists in agricultural and other biological fields.

Disciplinary boundaries are disappearing among the biosciences, as well as between bioscience and biotechnology. The stimulation of high-quality interdisciplinary research in agriculture must be a top priority.

The sophisticated technologies and products of research still emerge from the manners of science that have existed for hundreds and hundreds of years. Humberto Gerola and Ralph E. Gomory reported in a recent issue of Science:

Electronic communication, even when given away free, has not yet altered the fundamental way in which scientific work has been done. Face-to-face communication, so far, appears to be essential to scientific collaboration. . . . [It] has survived the change of



scale of science itself, from an activity carried out by a very small number of people to one involving thousands and thousands of researchers. It appears that it may well survive electronic communication.<sup>3</sup>

#### Recommendations

As the principal intramural research agency of the U.S. Department of Agriculture, the ARS has a long history of conducting research that has been translated, with outstanding success, into applications in seed, food, and fiber production. The committee strongly believes that the following 17 recommendations, addressing the larger issues of review, communications, leadership and staffing, organization, and scientific opportunities for researchers, will combine to promote the optimal climate necessary for creative, quality research within the ARS. This in turn will provide the basis for the future worldwide competitive advantage of U.S. production agriculture and agribusiness.

#### Periodic Outside Review

An advisory council consisting of 5 to 10 leading scientists in the research community and reporting directly to the ARS administrator should be created. The advisory council would provide a regular review of ARS research and, in addition, could communicate new directions in research and suggest strategies for guiding national research. This ARS Advisory Council (ARSAC) would have a rotating three-year membership and would delegate subcouncils as needed for review of all ARS programs on a three-year cycle. The subcouncils would be similar to the existing advisory committee at the ARS Plum Island Animal Disease Center in New York.

It is imperative that the members of the ARSAC be selected from among those national leaders in agricultural research who have a strong and active research background. In addition the individuals must possess a global view of agricultural science and technology.

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<sup>3</sup>Gerola, H., and R. E. Gemory. 1984. Computers in science and technology: Early indications. *Science* 225:11-18.

Their selection should be based primarily on these strengths, independent of their affiliation, be it academe, industry, or government. Members of any sub-council should come from a strong and active research base.

The ARSAC would act as a non-ARS source of information about state-of-the-art developments throughout the United States and the world for the ARS administrator and for other ARS leadership such as the National Program Staff. The ARSAC would suggest specific programs in basic agricultural sciences that will provide the highest dividends to U.S. agriculture. The council might also recommend program changes, including the initiation of new scientific efforts.

The precedent for such an outside advisory council has been set by scientific advisory boards to the National Bureau of Standards and the National Institutes of Health and by the National Science Board of the National Science Foundation. Many large corporations as well as smaller start-up companies have strong scientific advisory boards.

#### Leadership

The literature on leadership in organizations is dominated by the human relationship thesis that good leadership leads to high morale and that high morale leads to increased productivity of group members. The ARS must address its need for additional capable scientific leaders as laboratory chiefs. The committee particularly noted that both quality research and individual and group satisfaction were reflected by ARS laboratories supervised by dynamic and farsighted laboratory chiefs. These individuals should be selected first on the basis of their scientific excellence and second on the basis of their management potential.

The quality of laboratory chiefs is measured by the productivity and scientific excellence of their laboratory groups. To meet this responsibility laboratory chiefs need authority and flexibility in budgetary and personnel matters.

#### National Program Staff

The ARS National Program Staff, in addition to setting the long-term direction for the agency, has major control

of budget allocations for research. The committee perceived that communications between the National Program Staff and research scientists must be strengthened. One approach might be to assign laboratory chiefs temporarily to the National Program Staff on a rotating basis.

The committee recommends that the National Program Staff provide strong support for creative research in the laboratory while assuring the flexibility that is essential for pursuit of the most promising avenues of research. To accomplish this the National Program Staff not only must encourage open and frequent communications with ARS scientists but also must be receptive to the new ideas and new research directions emerging from those at the laboratory bench. What then becomes policy must be clearly communicated to all, management and staff.

Science is best and most aggressively pursued when supported by the stability and continuity of program objectives. During the past decade the ARS has undergone several reorganizations that have resulted in some abrupt and disruptive shifts in the direction of research programs. Not unexpectedly, continuity has faltered, to the detriment of long-range research direction. The National Program Staff, along with ARS management, must ensure that, if and when such events occur, program stability is preserved and that this reality is conveyed to the scientific staff.

#### New Centers

The committee was informed of the plans for the Plant Gene Expression Center to be established in collaboration with the University of California at Berkeley and the California Agricultural Experiment Station, and supports this novel plan. The new center, which will be located at the ARS Western Regional Center in Albany, California, offers a new opportunity for increased focus on basic research in the plant sciences. The committee members agree that the mission of this center should be to provide an understanding of gene structure and function with respect to key plant processes. This report offers examples of programs appropriate for the center.

The Plant Gene Expression Center will provide both the public and private sectors with the opportunity to convert the fundamental knowledge generated by the center to practical application. The long-term agricultural impact of the center will be to strengthen the base for U.S.

crop biotechnology. The committee recommends that a subcouncil of the ARSAC be created to provide scientific program advice for the center.

The ARS must constantly consider other new opportunities arising in the agricultural sciences and seek innovative ways such as this to exploit these opportunities.

#### Interdisciplinary Activities

The ARS has an unusually broad base and has excelled in many areas of traditional biology. The new biology now provides a set of techniques that are making possible advances in the understanding of major biological systems and processes. This understanding may then be translated to new technologies. Central to the successful use of these newer techniques is the promotion of interdisciplinary research. The committee recommends that the scientific and managerial leadership of the ARS seek ways to facilitate interdisciplinary activities. The ARS appears to be in a most fortunate position to pursue such approaches, since the agency is not constrained by the departmentalized disciplinary organization that is characteristic of academic institutions.

#### Consolidation

The committee has noted that there is inadequate communication and duplication of scientific efforts at a number of the 147 ARS research centers. The committee recognizes that multiple geographic research locations are important to agricultural research, but also believes that the number of sites is too large and must be reduced to create a critical mass--more effective research groups--at fewer sites. Although modest duplication may be beneficial to science, excessive duplication is not an effective use of limited economic and scientific resources.

The committee recommends three approaches. In one, sites specializing in similar research areas would be consolidated to give a more effective concentration in a scientific area. In another, the smaller numbers of ARS scientists at some centers would be coupled with strong academic groups to achieve the same end result. Increasing scientific sophistication requires that some of

the 147 centers be consolidated and/or located on a university site to make best use of facilities, to complement areas of expertise, and to increase the opportunity for additional interdisciplinary interactions. In the third case, smaller research groups having scientific missions that are no longer critical or of high priority would be discontinued.

The committee recommends that the ARSAC be asked to make specific recommendations for the consolidation of scientific programs within the ARS.

### Leveraging

In general, one of the ARS's outstanding advantages is that a large capital investment in facilities has already been made. Program changes and the addition of crucial staff members will yield a significant positive effect. The addition of a number of people with newer biology skills to the current ARS scientific staff, with its substantial base in the more traditional biology, could provide a strong synergistic effect. This would ensure the ARS future status as a strong world leader in many areas essential to advances in the agricultural sciences and technology.

### Postdoctoral Program

The ARS has responded to the demands of the new biology by creating a special postdoctoral program and by streamlining the hiring process for those temporary employee appointments. (Twenty-five researchers were hired in fiscal year 1984, and future additions are anticipated.) The committee recommends that the ARS aggressively expand its newly adopted program, with the goal being a steady state of about 750 nontenured positions dedicated to postdoctoral fellows and senior staff fellows. These nontenured positions should be distributed throughout the most productive basic research programs of the ARS. The resulting ratio would be less than one nontenured position per tenured basic research scientist.

This type of program is virtually the best single mechanism for bringing new techniques, new capabilities, and new ideas into the ARS. The postdoctoral appointments should be for a minimum of two years, with an

option to extend the position on a yearly basis, to a maximum of five years.

Each research scientist should be responsible for the recruitment and selection of postdoctoral candidates to fill positions in his or her laboratory. The hiring period should be as short as possible. Even the approximately 150-day hiring period that will result from the recent ARS plan to reduce hiring time is much too long for top-ranked postdoctoral candidates to wait for job confirmation.

Successful implementation of a growing postdoctoral program would assure the ARS stature as a major contributor to U.S. competitiveness in providing trained people for the agricultural sciences, much as the National Institutes of Health is viewed as a provider of trained personnel for the medical research community.

#### Appointment of New Staff

The ARS possesses a well-recognized procedure for internal evaluation of tenured staff that has been the model for other federal agencies such as the Department of Defense and the Department of Energy. The current policy of evaluating employees within one year from the time of hiring, however, does not allow for an adequate assessment of an individual's scientific productivity or potential. The committee recommends that the decision to grant tenure should be made upon review after five years, for Ph.D.-level basic research scientists. The committee also recommends that the decision to offer a permanent appointment include an appraisal of the candidate's scientific contributions by outside scientists in the candidate's field. Currently, nearly 100 percent of those individuals evaluated one year after the time they were hired received tenure. With the institution of a larger postdoctoral program and a rigorous outside appraisal system, the committee expects that this figure might drop significantly.

#### Budget

The new biology requires special equipment and expendables, such as restriction enzymes, other specialized biologicals, and tissue culture supplies, that are relatively high in cost. This highly intensive, equipment-

oriented research does not diminish the importance of ideas; however, to carry out new ideas, the ARS must plan for the cost of equipment and maintenance contracts. The committee noted that in many ARS centers nearly 90 percent of the total budget was designated for salaries, and thus recommends that this figure be reduced to approximately 75 percent. In instances where purchase of materials is particularly critical to the maintenance of high-quality research, funds designated for salaries might be as low as 60 percent of the total budget.

The flexibility to alter direction in exploratory research is critical to scientific excellence. The committee recognizes that long-term financial planning is essential, but budgets must be shaped with an inherent flexibility to allow for redirection of research into unexpectedly promising new scientific areas. The ARS should designate approximately 10 percent of the total budget of centers as flexible funds to support meeting attendance and research-related travel, and perhaps more importantly, to allow for a rapid response to significant findings that require a change in research direction. Continued scientific oversight would provide review of the effective use of these discretionary funds.

#### Support Staff

The ARS should continuously monitor its need for support staff (technicians), particularly with the addition of any new programs. The availability of a substantial number of support staff trained at the bachelor's or master's degree level will allow the ARS scientific staff to compete effectively with researchers throughout the world.

Many areas of the new biology are highly labor-intensive and require skills in monoclonal antibody production, protein sequencing, and oligonucleotide synthesis. The ARS must always plan for the addition of some special research capabilities and instrumentation as science advances. Centralized facilities that provide special assistance or technical service and are accessible by other sites might be most cost-effective.

#### Sabbaticals/Retraining

The ARS should encourage its scientists to take sabbaticals to maintain skills at the leading edge of

science. The committee recognizes that sabbaticals can be expensive to the agency, but also believes that it is not cost-effective to support scientists who are not trained to utilize current techniques. Funding from outside the ARS should be used for sabbaticals when possible.

The committee noted that a small number of ARS centers supported very active retraining programs that involved almost all of the scientific staff members. The ARS should take advantage of the opportunity to enhance the capabilities of some of its scientists by retraining them in newer research-oriented methods.

#### Scientific Meeting Attendance

Attendance at national and international meetings by ARS scientists is critical; face-to-face communication, as noted earlier in this chapter, is still the most effective method for the exchange of ideas in the scientific arena. The committee believes most strongly that the ARS must give a higher priority to allocation of funds for this aspect of scientific exchange and growth. Adequate travel resources should be available for invited ARS speakers and organizers, session chairmen, and select research scientists. The flexibility to respond quickly to travel approval requests is essential.

To promote scientific exchange and help alleviate budget constraints, ARS scientists should be encouraged to accept outside travel support when available.

#### Publications

Limited peer review of papers within the ARS laboratory, combined with a routine scientific journal review, will bring research results into publication more quickly. A protracted internal publications approval process is unnecessary.

The quality of publications is an important measure of a scientist's productivity. The committee noted that the method of awarding merit points in order of authorship on publications--a technique employed at some ARS centers--can lead to inappropriate orders of authorship and can fail to reflect the true scientific contribution of the individual. Such a merit point system could inhibit collaborative work, which is the basis for progress in the new biology.



### University Relationships

The committee noted that several strong relationships have been established between the ARS and universities, most recently that between the ARS center at Albany and the University of California at Berkeley in the development of the Plant Gene Expression Center. ARS/university associations or relationships can provide a valuable source of information and inspiration as well as feedback and critical review. Those ARS centers located on or very near university campuses appear to profit from the richness of such an exchange of information and participation of researchers. The university can contribute to and strengthen such relationships by awarding adjunct professorships where appropriate.

The establishment of additional relationships between strong university groups and select ARS scientists is encouraged. Such relationships involving even just a few ARS scientists can bring that number to the essential critical mass needed for the pursuit of creative research. The mutual scientific benefits of continuing such relationships should be evaluated on a regular basis.

### Industry Relationships

The ARS must begin to explore research relationships in biotechnology with industry, just as many universities have recently begun to do. These may range from seminars or laboratory visits to cooperative research. All programs must be open to the scientific community.

The ability of ARS scientists to supplement their incomes with honoraria from industry-sponsored public seminars would help alleviate the constraining salary cap that now may preclude the ARS from hiring or retaining the best scientists. The committee understands that such an approach is currently being used by the National Institutes of Health.

### Public Relations

The ARS, along with industry, universities, the states, and private foundations, must make an effort to educate the public about the importance of agriculture to the health of the U.S. economy and to that of its people. Programs such as the U.S. Department of

Agriculture's "Agriculture in the Classroom" will broaden the understanding of how high-quality foods are brought from the farm to the consumer at relatively low cost, and perhaps stimulate young people to pursue careers in the agricultural sciences. All individuals within the ARS have a responsibility to communicate both the opportunities and the need for adequately funded support of agricultural research and technology.

### Conclusion

The committee made note of the inherent assets of the ARS--the superb facilities at some of the research centers; the network of centers that offers the ideal foundation for rapid communication throughout the system; its basic structure as a potential training ground for new scientists; and the opportunity to stress long-range, high-risk, high-reward research without the more intense pressures of product development and profit. It is of utmost importance that these strengths be maintained and perhaps amplified.

The committee was very encouraged by the major effort of the administrator to position the ARS as the leader of world agricultural science and technology. The members hope that this report will assist him in this challenging endeavor.

Coupled with these strengths of the ARS, the factors contributing to an optimal climate for research, as described in this report, will enable the ARS to provide a strong basis for continuing progress in U.S. agriculture. The ARS, as with the entire research establishment, can most effectively adapt to the rapid pace of scientific developments and maintain research leadership by creating a competitive yet rewarding research environment that attracts and encourages the most creative and productive scientists. This foundation will be critical in establishing strong competitive programs, in both U.S. agricultural production and support industries, that will successfully meet the ever-increasing challenges of world agriculture.

# Appendix

## Agricultural Research Service Laboratory Groups Visited

### Albany, California

#### Western Regional Research Center

Biocommunication Chemistry Research  
Biological Control of Weeds Research  
Cereals Products Research  
Chemurgy Research  
Food Proteins Research  
Natural Products Chemistry Research  
Nutrients Research  
Plant Physiology and Chemistry Research  
Plant Protection Phytochemistry Research  
Toxicology and Biological Evaluation Research

### Ames, Iowa

#### National Animal Disease Center

Bacteriological and Mycological Laboratory  
Pathological Laboratory  
Phytopathological Laboratory  
Virological Laboratory

### Athens, Georgia

#### Southeast Poultry Research Laboratory and the Richard B. Russell Agricultural Research Center

Animal Physiology Research Unit  
Food Protection and Processing Research Unit

Meat Quality Research Unit  
 Poultry Disease Unit, SPRL  
 Poultry Genetics Unit, SPRL  
 Poultry Physiology Unit, SPRL  
 Toxicology and Biological Constituents  
 Research Unit

Beltsville, Maryland

Beltsville Agricultural Research Center

Agricultural Environmental Quality Institute  
 Biologically Active Natural Products  
 Laboratory  
 Insect Reproduction Laboratory  
 Organic Chemical Synthesis Laboratory  
 Pesticide Degradation Laboratory  
 Soil Nitrogen and Environmental Chemistry  
 Laboratory  
 Weed Science Laboratory  
 Animal Parasitology Institute  
 Biosystematic Parasitology Laboratory  
 Helminthic Disease Laboratory  
 Protozoan Disease Laboratory  
 Animal Science Institute  
 Animal Improvement Programs Laboratory  
 Avian Physiology Laboratory  
 Milk Secretion and Mastitis Laboratory  
 Nonruminant Animal Nutrition Laboratory  
 Reproduction Laboratory  
 Ruminant Nutrition Laboratory  
 Horticultural Science Institute  
 Florist and Nursery Crops Laboratory  
 Fruit Laboratory  
 Horticultural Crops Quality Laboratory  
 Vegetable Laboratory  
 Plant Genetics and Germ Plasm Institute  
 Seed Research Laboratory  
 Tobacco Laboratory  
 Plant Physiology Institute  
 Light and Plant Growth Laboratory  
 Nitrogen Fixation and Soybean Genetics  
 Laboratory  
 Plant Hormone Laboratory  
 Plant Stress Laboratory  
 Tissue Culture and Molecular Genetics  
 Laboratory

Plant Protection Institute  
Bioenvironmental Bee Laboratory  
Plant Pathology Laboratory

Clay Center, Nebraska

U.S. Meat Animal Research Center

Genetics and Breeding Research  
Meats Research  
Nutrition Research  
Reproduction Research

College Station, Texas

Cotton and Grain Crops Genetics Laboratory  
Cotton Insects Research Laboratory  
Cotton Pathology Research Laboratory

Denver, Colorado

Arthropod-Borne Animal Disease Laboratory

Foreign Animal Disease Control Unit  
Livestock Insect Control Unit

East Lansing, Michigan

North Central Region, Lake States Area

Avian Leukosis Research Laboratory

Fargo, North Dakota

Metabolism and Radiation Research Laboratory

Gainesville, Florida

Biological Pest Control Research Laboratory  
Environmental Physiology Research Laboratory  
Insects Affecting Man and Animal Research  
Laboratory  
Insect Attractants, Behavior, and Basic Biology  
Plant Science Research Laboratory  
Soil and Water Research Laboratory

Madison, Wisconsin

Bee Management and Entomology Research Unit  
Plant Disease Resistance Research Unit

Manhattan, Kansas

U.S. Grain Marketing Research Laboratory

Pasadena, California

Fruit and Vegetable Chemistry Research

Peoria, Illinois

Northern Regional Research Center

Cereal Science and Foods Laboratory

Cereal Protein Research

Fermentation Laboratory

Microbial Chemistry Research and Agricultural  
Research Culture Collection

Horticultural and Special Crops Laboratory

Plant Biochemistry and Photosynthesis

Oilseed Crops Laboratory

Biochemistry and Biophysical Properties  
Research

Plum Island, New York

Plum Island Animal Disease Center

Biochemical and Biophysical Research Laboratory

Cytological Research Laboratory

Immunological Research Laboratory

Pathobiological Research Laboratory

Raleigh, North Carolina

Environmental Physiology Research Laboratory

Plant Science Research Laboratory

Soil and Water Research Laboratory

Riverside, California

U.S. Salinity Laboratory

Plant Sciences Research

Soil Chemistry Research

Soil Physics Research

St. Paul, Minnesota

Cereal Rust Research

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**Plant Sciences Research  
Soil and Water Management Research**

**Urbana, Illinois**

**Photosynthesis Research  
Soil, Water, Plant Research  
Soybean Breeding and Production Research**

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