

**DIVISION OF BIOLOGICAL ENERGY RESEARCH
OFFICE OF BASIC ENERGY SCIENCES**

**ANNUAL REPORT AND SUMMARIES OF
FY 1980 ACTIVITIES**



**U.S. DEPARTMENT OF ENERGY
OFFICE OF ENERGY RESEARCH**

AUGUST 1980

BACKGROUND AND INTRODUCTION

The Biological Energy Research (BER) program was established during FY 1979 as a new program within the Office of Basic Energy Sciences. The foundation of the program was a group of ongoing projects funded at approximately four million dollars which had previously been supported in the Office of Health and Environmental Research under the Assistant Secretary for Environment in the Department of Energy. Most of the projects which were transferred to constitute the new BER program were in the basic plant sciences and it was not until FY 1980 that additional funds became available with which to initiate new projects.

The principal objective of the BER subprogram is to provide the biological foundation of basic information for the Department's efforts for biomass production of fuels and chemicals, microbiological transformation of organic materials for conservation and using biological systems for resource recovery. The program has two facets, botanical investigations and microbiological studies. The research is aimed at developing a broad, intensive, fundamental understanding of the biological factors involved in plant biomass productivity, conversion of biomass and other organic materials using microorganisms into fuel and chemicals, and with ideas for using biological systems for sparing energy resources (conservation).

While other DOE programs emphasize near- to mid-term research and development needs, the BER program is oriented towards longer-term and fundamental research. BER is the only basic biological program in the Federal Government specifically aimed at studying biological systems in energy matters. The BER program aims at developing broader and deeper understanding of numerous aspects of the mechanisms involved in: limitation of green plant productivity, adaptability for growth and productivity of plants under conditions of environmental stress, the biological regulatory processes which determine how plants synthesize and distribute reduced carbon compounds both quantitatively and qualitatively, and the biochemical expression of genetic information. Emphasis is also placed on other topics bearing on improving plant productivity for fuels and chemicals.

The microbiological research also places emphasis on development of understanding of fundamental mechanisms. Much attention is to be given studies on anaerobic microorganisms involved in fermentations. In particular, the biochemical pathways of degradation of abundant substrates such as cellulose, hemicelluloses and lignins are given attention as are the various follow-on fermentation metabolic

pathways and their control. The genetics of anaerobic microorganisms is another key element. Such phenomena as thermophily, end product inhibition and other regulatory aspects of fermentations are also to be given emphasis. Microbial degradation and conversion mechanisms involving hydrocarbons and other carbonaceous fuel or chemical materials are to be included.

The topics represented above in the botanical and microbial areas are only examples of BER interests and are not all inclusive.

The BER program depends almost exclusively upon the submission of unsolicited proposals. The rationale is to encourage communication through informal proposals and discussions to convey interests. However, the individual investigator must be the originator of the research ideas, the approaches and plans. BER only indicates the broad areas of interest and depends on the responsiveness of the scientific community to those interests and needs.

The FY 1980 budget for the BER program amounted to six million dollars in operating funds and three-hundred thousand dollars in equipment monies. Approximately two million dollars was available for new or enhanced initiatives during this fiscal year. In order to bring attention to the program, announcements were sent to several relevant professional society newsletters requesting publication of the BER program interests in abbreviated form and indicating that BER would consider unsolicited research proposals. During this period of program development, numerous inquiries have been received from many universities, research institutes, and other institutions. In order to be most economical of time and effort the submission of informal proposals by prospective investigators was the principal means of determining program interest. The formal research proposals which were received followed two deadlines for submissions, one in the late summer of 1979 and the other in mid-winter 1980. About 100 formal proposals were submitted during the year with a total request of almost \$9,000,000 for the first year of support. Twenty-six new research projects were funded (average \$68,131 annually) along with several small awards for conferences and workshops.

The proposals were reviewed by scientific peers. Two-hundred seventy-seven individual scientists in institutions both in the U. S. and abroad assisted with

the scientific evaluations. The proposals were reviewed by scientists to whom the proposals were sent in the mails and also by ad hoc evaluation panels. The panelists were asked to take into consideration not only their own impressions of the proposals, but also the mail reviewers' comments and criticisms which were provided anonymously after their own evaluation had been made. Several review procedures were tried during this period in an effort to achieve a critical, fair and efficient system of obtaining evaluations of the proposals submitted. It is felt that the technical review process did satisfy the program needs in terms of insuring initiation of high quality research in appropriate areas. The BER program, like others, was not able to fund all of the meritorious proposals offered. A breakdown of the funding pattern for FY 80 is indicated below. A major fraction (86%) of the new initiatives were sponsored in university laboratories and other educational institutions.

Breakdown by type of institution where BER funds were spent in FY 80:

| | <u>Amount</u> | <u>Percent of Total</u> |
|--|---------------|-----------------------------|
| University research contracts | \$2,459,556 | 41 |
| University on-site facilities (principally Michigan State University) | 1,641,000 | 27 |
| National Laboratories (Brookhaven National Laboratory, Lawrence Berkeley Laboratory) | 1,515,000 | 25 |
| Industrial research institute contracts | 229,444 | 4 |
| Solar Energy Research Institute | 117,000 | 2 |
| Miscellaneous | <u>38,000</u> | 1 |
| | \$ 6,000,000 | |

In FY 1980, in an effort to address the important question of research in need of additional support, the BER program sponsored several workshops bearing on long-term energy questions of a biological nature which will require more attention as the program moves ahead:

1. Biological Energy Research Workshop - November 2-4, 1979, Belmont, MD (through the Solar Energy Research Institute)
2. Plant Molecular Biology Workshop, February 29 - March 2, 1980, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (with NSF)
3. The Biological Response of Higher Plants to Environmental Stress, March 26-28, 1980, University of Minnesota, St. Paul, MN
4. The Genetics of Obligate Anaerobic Microorganisms Workshop, April 22, 1980, Chicago, Illinois
5. Conference on the Energetics of Biological Fixation of Dinitrogen, April 27-30, 1980, Michigan State University, East Lansing, MI

In addition, the BER program, in concert with the National Science Foundation, sponsored a symposium entitled "Symposium on Genetic Engineering of Osmoregulation: Impact on Plant Productivity for Food, Chemicals and Energy" at Brookhaven National Laboratory, November 4-7, 1979.

The BER program is in the early stages of development and an optimal program balance has not yet been achieved. It is anticipated that there will be continuing modifications in the makeup of the program with changing emphases as a reflection of scientific progress and evolving energy supply situations.

The success of the BER program will ultimately be measured by several criteria. The first must be scientific achievement in elucidating complex biological phenomena. The second is how rapidly and effectively there is a transfer and utilization of the understanding generated by the research in attacking significant energy related problems. The third is the most efficient use of the resources available to BER in accomplishing the first two objectives. It is recognized that in accomplishing these objectives, the cooperation and involvement of scientists in many sectors, the universities, the national laboratories, industrial laboratories, federal laboratories, and others will be essential. Their interest and assistance has been most appreciated in the past and will be in the future.

In the following pages research project summaries are provided for each of the efforts supported in FY 80. Projects are funded normally on an annual basis with provision for longer funding depending on the nature of the project. In most cases projects are given peer review every third year.

The data provided for the individual project contracts indicates the funding (direct and indirect costs) for one year unless otherwise noted. With respect to national laboratory and other "on-site" funding the total levels are provided for the laboratory in question. The sub-projects within the lab have the scientific person years accompanying the individual summaries to indicate the levels of effort. Any reader with questions about the details of an individual project may contact the particular investigator involved. Questions about the overall Biological Energy Research program should be directed to:

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1. BATTELLE COLUMBUS LABORATORIES
Columbus, Ohio 43201

COMPLEXES OF CHLOROPHYLL DERIVATIVES WITH
HEME APOPROTEINS

\$99,445
(15 months)

Robert M. Pearlstein
Organic & Polymer Chemistry Section

Chlorophyll (Chl) in vivo and in isolated lipoprotein complexes has spectroscopic and photochemical properties that distinguish it from monomeric Chl in a simple solution. Present ideas regarding the origin of these distinct properties tend to emphasize Chl-Chl interactions, rather than the interactions between Chl and the protein to which all in vivo Chl is now known to be bound. One reason for this emphasis is that, in any native Chl-protein complex so far isolated, there has been at least two Chl molecules, some of the interactions between which cannot be easily separated from those between Chl and protein. We have reported the first 1:1 pigment-protein complex--formed by interaction of the water-soluble Chl derivative, chlorophyllin (Chln), with the apoprotein of myoglobin (Mb)--that undergoes photoconversion to a long-wavelength (690 nm) absorbing form resembling in vivo Chl. We propose to determine to what extent Chln-apoMb, and other 1:1 complexes of a Chl derivative with a heme apoprotein, can serve as models for native Chl-protein complexes. Experiments are planned in six areas: 1. Single crystal studies. 2. Photochemically-induced electron transfer from Chln-apoMb complexes to suitable acceptors. 3. Formation and characterization of complexes of other Chl derivatives with apoMb or other heme apoproteins. 4. Studies of the mechanisms that produce the spectral transformations in Chln-apoMb complexes. 5. Exploration of energy-storing reactions photocatalyzed by Chln-apoMb complexes. 6. Fluorescence studies. Preliminary efforts in the first area have just yielded the first large crystals (0.2-0.7 mm long, 0.1-0.2 mm wide) of Chln-apoMb complexes.

2. BRANDEIS UNIVERSITY
Waltham, Massachusetts 02154

EFFECT OF LIGHT ON RESPIRATION AND DEVELOPMENT
OF PHOTOSYNTHETIC CELLS

\$43,000

Martin Gibbs
Institute for Photobiology

Anaerobically adapted algae can photooxidize H_2O to H_2 and O_2 coupled to the oxidation of reduced carbon compounds to H_2 and CO_2 . The latter oxidation also occurs in the dark. The term "chloroplast respiration" has been proposed to account for the fact that the chloroplast is an electron donating organelle resulting in the formation of H_2O_2 , ammonia and H_2 , respectively. In this contract, we are primarily concerned by the means that endogenous polyglycan and exogenous glucose and acetate stimulate H_2 photo- and dark H_2 evolution. We intend to determine the potential role of the citric acid and glyoxylate cycles in anaerobic oxidation. We also plan to study photoreduction and the oxy-hydrogen reaction in these H_2 -adapted cells. We will utilize intact cells of Chlamydomonas and Chlorella. We plan to initiate preparation of protoplasts and chloroplasts from these unicellular algae.

The substrate for photorespiration is glycolic acid. The chloroplast produces in the light a considerable amount of H_2O_2 . We plan to investigate with the spinach and Sedum chloroplast the peroxidation of glycolaldehyde-thiamine pyrophosphate as well as other substrates as potential sources of glycolic acid.

3. UNIVERSITY OF CALIFORNIA
Santa Cruz, California 95064

ORGANELLES AND THE REGULATION OF PLANT METABOLISM

\$46,266

Harry Beevers
Department of Natural Science

We will investigate the metabolism of plant tissues in low O_2 environments. Plants show different abilities to withstand periods of anaerobiosis; some like rice can show growth under those conditions, most are killed when deprived of O_2 although various degrees of resistance are found. The metabolic bases for these differences are not established. We will investigate rates of ethanol and lactate accumulation in organs subjected to anaerobiosis, and determine the levels of added ethanol which are inhibitory. Balance sheets will be constructed to show how sugars are used in rice and other seedlings, to see how the rice is able to show superior resistance to anoxia.

4. UNIVERSITY OF CALIFORNIA
Los Angeles, California 90024

ENERGY CAPTURE AND USE IN PLANTS AND BACTERIA \$71,734
Paul D. Boyer
Molecular Biology Institute

The principal objective of this work is to find how the chemical substance ATP is made in biological systems from energy provided by oxidations or from light. More ATP participates in chemical reactions, on a molar or weight basis, than any chemical compound in the world except water. How this is produced in biological systems remains one of the foremost challenges in biochemistry.

We use chloroplast thylakoids isolated from plants, submitochondrial particles from heart and chromatophores from photosynthetic bacteria to test the hypothesis, developed principally in our laboratory, that ATP synthesis is achieved through energy linked changes in the binding of substrates and products. More specifically, our evidence indicates that energy is used to promote the binding of P_i and ADP at catalytic site in a manner capable of forming bound ATP without further energy input, and in the release of preformed ATP from another catalytic site.

Approaches include the use of rapid mixing and quenching techniques, measurement of the amount and labeling of catalytic nucleotides, and the extent and patterns of ^{18}O distribution in products.

5. UNIVERSITY OF CALIFORNIA
Davis, California 95616

FLUORESCENCE PHOTOBLEACHING MEASUREMENTS OF PLANT MEMBRANE VISCOSITY: MECHANISMS OF RESISTANCE TO ENVIRONMENTAL STRESS \$35,000

R. W. Breidenbach, Plant Growth Laboratory
D. W. Rains, " "
M. J. Saxton, " "

Since temperature and salinity are among the most important factors limiting plant productivity, an understanding of the mechanisms of resistance to chilling and salinity will be useful in developing new plant varieties for production of food, fuel, and chemicals.

The proposed experiments will examine the role of the cell membrane in stress resistance, by means of fluorescence photobleaching recovery measurements of lateral diffusion constants of membrane proteins. The membrane viscosities for chilling-sensitive and chilling-resistant plants will be determined at various temperatures, to see whether the viscosity in chilling-sensitive plants increases abruptly at the critical temperature for chilling injury. Membrane viscosities will also be compared for salt-tolerant and salt-intolerant lines of alfalfa and barley to examine the role of the membrane in salt resistance. The use of photobleaching recovery of labeled proteins to measure viscosity is particularly appropriate since many proposed cellular mechanisms are directly dependent on lateral motion of membrane proteins.

6. UNIVERSITY OF CALIFORNIA
La Jolla, California 92093

BIOLOGICAL ACTIVITY OF MOLECULAR HYDROGEN: \$75,000
ENHANCEMENT AND STABILIZATION OF HYDROGENASES
M. D. Kamen, Department of Chemistry
N. O. Kaplan, " "
R. G. Bartsch, " "

Our ultimate objective is to construct from chloroplast fragments and purified hydrogenase a practicable bisolar reactor for the photolysis of water to fuel-grade hydrogen gas. Our efforts are now concentrated on a search for a source of a stable hydrogenase, on methods for purifying the enzyme in quantity, on studies of the properties of the purified enzyme and on methods for increasing the stability of the enzyme when incorporated into a reactor.

Of the more than 20 different bacteria and algae examined, two, Desulfovibrio vulgaris and Alcaligenes eutrophus, were found to be the most suitable sources, yielding 100-200 mg of soluble, stable enzyme per kg of cells. The hydrogenase of the first organism has been sufficiently purified to yield crystallize protein which is to be used for x-ray crystallographic study of the molecular structure, the better to understand the mechanism of action of the enzyme with bioreactor components. Methods for protecting oxygen-labile hydrogenase have been devised, using high ionic strength or multicharged solvent to minimize the solubility of molecular oxygen accessible to the protein.

The light-driven photolysis of water to combustible products in a reactor using catalysts derived from plants and bacteria has been demonstrated on a micro scale in our laboratory and elsewhere.

7. UNIVERSITY OF CALIFORNIA
Irvine, California 92717

BIOENERGETICS OF SALT TOLERANCE

\$116,000

Janos K. Lanyi, Department of Physiology
Lester Packer, UC-Berkeley
Stephen H. White, UC-Irvine

Information now available from halophilic and halotolerant bacteria suggests that the cytotoxic effect of salt is based primarily on the disruption of macromolecular structures, e.g., enzymes and ribosomes, by sodium ions. Halobacteria overcome this largely by coupling a powerful sodium ion extrusion system to the gradient for protons developed during light energy conversion by bacteriorhodopsin or electron transport. This mechanism is based on proton/sodium antiport. In the halobacteria a second mechanism for sodium extrusion has been recently postulated, based on a light-energy converter that will directly transport sodium across membranes. Exit of sodium ions in different halotolerant microorganisms is followed by either potassium ion uptake, the synthesis of organic osmoregulatory substances, or both. We intend to describe the transport mechanisms involved in bacterial systems and in halophytic plant cells, and to isolate and characterize the transport proteins at the molecular level.

The goal of this research is to aid the development of highly salt-tolerant crops by identifying and describing those evolutionary adaptive features which have enabled halotolerant and halophytic organisms to effectively remove sodium from their cytoplasm, and thus overcome the deleterious effects of salt.

8. UNIVERSITY OF CALIFORNIA
Los Angeles, California 90024

METHANOGENESIS FROM ACETATE, A KEY INTERMEDIATE \$61,357
IN NATURE

Robert A. Mah
Division of Environmental & Nutritional Sciences
School of Public Health

The production of biogas (methane) from various types of organic feedstocks including municipal solid wastes, livestock and human wastes, agricultural crop residues, etc. is dependent on a complex microbial fermentation by unknown mixtures of bacteria comprised of non-methane-producing and methane-producing (methanogenic) organisms. About 70 percent or more of the methane produced in any biogas fermentor by the methanogenic bacteria comes directly from an intermediate compound called acetic acid which is formed from the degradation of all organic compounds by non-methanogenic bacteria. However, most of the microbiological knowledge of methane formation comes from pure culture studies on non-acetate using methanogens which metabolize hydrogen and carbon dioxide to make methane. This proposal focuses on the less well described acetate-using methanogenic bacteria which produce most of the methane in biogas fermentations. The objectives are to isolate additional strains/species of acetate-splitting methanogens, characterize them by studying their metabolism and nutritional requirements, and to develop mutant strains as a base for future genetic studies. Since these acetate-using methanogens are the most important ones in biogas formation, this study should lead to a better understanding of this group of organisms and potential applications for improving the formation of methane.

9. UNIVERSITY OF CALIFORNIA
La Jolla, California 92093

SELECTION OF MUTANTS INCREASING THE RATE
OF FERMENTATION IN YEAST

\$65,000

Christopher Wills
Department of Biology

We have developed a selection system for producing modifications of the fermentative yeast alcohol dehydrogenase that affect turnover number, binding constants and subunit cooperativity in a variety of ways. Allyl alcohol selection on petite cells results in a high proportion of structural gene mutations at the ADH-I locus affecting these properties. The source of the resistance to allyl alcohol is an enzyme-mediated change in the redox balance of the cell. Two of the amino acid substitutions responsible for this resistance have been identified.

Some of the mutants isolated using this selective system show a shift in the direction of increased ethanol production under steady-state conditions. Selection schemes employing allyl alcohol and other compounds will be applied to the development of mutant enzymes with an increased turnover number and with an increased ability to push the reaction in the direction of ethanol. Mutants derepressed for ADH at high substrate concentrations will also be selected for.

The amino acid substitutions in the mutant enzymes will be determined. The physiology of the mutants will be investigated under a variety of steady-state conditions. The ability of the most promising mutants to increase the rate of production and total amount of ethanol produced under batch and continuous-flow fermentation systems will be investigated. Selection experiments involving recombination of known mutants under strongly selective conditions will be carried out to produce enzymes that differ more from the wild type in their properties than do single step mutants.

10. COLORADO STATE UNIVERSITY
Fort Collins, Colorado 80523

MICROBIAL PRODUCTION OF ALIPHATIC HYDROCARBONS \$66,602
Thomas G. Tornabene
Department of Microbiology
(Investigator has moved to Solar Energy Research Institute,
Golden, Colorado)

The proposed research will establish a basis for the development of a microbial system for the commercial production of oily hydrocarbons. The program is divided into three parts: those dealing with studies on hydrocarbon composition of cells, the effects of physical-chemical factors on efficiency of hydrocarbon production, and the genetic basis of hydrocarbon production. Within these programs is the screening of microorganisms for those that have the highest hydrocarbon production potential. From these studies we hope to establish a systematic scheme for future screening of hydrocarbon producing microbes for the optimum hydrocarbon producers. Also within this program are studies that will determine the biosynthetic mechanism and its regulation. The development of the hydrocarbon biosynthesizers for industrial production will then depend on the studies conducted on the genetic basis of hydrocarbon production.

In this project, we are essentially looking for a microbial system which will grow on commercially suitable media that has an unusually high lipid and hydrocarbon producing capacity and which actually leak or pump the hydrocarbons into the growth medium. These events have already been individually observed in our laboratory for specific micrococcal strains and we feel that with continuous studies the organism will be found or obtained that has all these features in one cellular system.

11. UNIVERSITY OF COLORADO
Boulder, Colorado 80309

STUDIES OF PLANT CELL WALLS AND OF PLANT-
MICROBE INTERACTIONS

\$197,000

Peter Albersheim, Department of Chemistry
Barbara Valent, " "
Alan Darvill, " "
Michael McNeil, " "

1. Host-Pathogen Interactions. We are studying the general defense mechanisms of plants and, in particular, have isolated and characterized elicitors of phytoalexin production in plants. The elicitors, which affect a wide variety of plants, are found in the cell walls or cell surfaces of many microorganisms. The laboratory is also attempting to identify those factors which determine race-specific host-pathogen interactions in gene-for-gene systems. 2. Host-Symbiont Interactions. We are investigating various Rhizobium-legume interactions trying to determine the molecular constituents of these organisms which interact to account for the high degree of host selectivity exhibited. This research includes an attempt to explain why legumes do not reject as foreign organisms symbiont Rhizobium. 3. Structure and Function of Cell Walls. We are involved in detailed structural analysis of the primary cell walls of plants. 4. Methods for Structural Characterization of Complex Carbohydrates. We have recently reported a combined rapid and sensitive HPLC, GLC, and MS method for structural characterization of complex carbohydrates.

12. CORNELL UNIVERSITY
Ithaca, New York 14853

STUDIES OF PHOTOSYNTHETIC ENERGY CONVERSION

\$65,530

Roderick K. Clayton
Division of Biological Sciences

Having pioneered the isolation and characterization of the photochemical reaction centers that initiate the conversion of light to chemical energy in photosynthetic organisms, we have gained a detailed knowledge of the earliest physical and photochemical processes of photosynthesis by studying the behavior of these reaction centers. Based on our findings, workers in other laboratories have begun to succeed in constructing artificial reaction centers; molecular complexes that duplicate the events of natural photosynthesis. These advances may help to develop methods of solar energy conversion based on photosynthesis. We have begun to elucidate the detailed structures of the natural reaction centers, through measurements with polarized light. We are also extending our knowledge of secondary electron transfer processes that follow the primary photochemistry and lead to the formation of ATP. These studies have been made principally with photosynthetic bacteria.

13. UNIVERSITY OF GEORGIA
Athens, Georgia 30602

MICROBIAL OXIDATION OF AROMATIC SULFUR AND
NITROGEN CONTAINING CONSTITUENTS OF FOSSIL FUELS

\$74,141

William R. Finnerty
Department of Microbiology

Petroleum, coal liquids and shale oil is characterized by the presence of significant quantities of sulfur- and nitrogen-containing aromatic compounds varying in concentration from 0.025-6.0%. The desulfurization and denitrogenation of fossil fuels involves chemical and physical processes such as hydro-desulfurization that are costly and generally inadequate for coping with an ever increasing dependence on such fuels. A biological approach to the desulfurization and denitrogenation of fossil fuels merits further consideration and basic research development as a potential energy-related support technology. The biological process involves the use of select microorganisms to attack specific sulfur- and nitrogen-containing constituents present in fossil fuels and the conversion of these constituents to easily removable water-soluble organic products. Several bacterial species have been isolated which attack specific sulfur- and nitrogen-containing aromatic compounds present in petroleum (dibenzothiophene and carbazole). These biological oxidations require aerobic conditions and effect the conversion of S- and N-constituents to water soluble products with the S and N remaining bound in covalent form to the organic moiety. These bacterial isolates are used solely as specific biological catalysts to perform highly specialized reactions. Studies into the basic physiology and biochemistry of these microorganisms is described with special emphasis on the relationship of these physiological attributes to plasmids. The functional role of plasmids in this biological process offers future possibilities for gene engineering and strain improvement.

14. UNIVERSITY OF GEORGIA
Athens, Georgia 30602

ENVIRONMENTAL STRESS MEDIATED CHANGES IN
TRANSCRIPTIONAL AND TRANSLATIONAL REGULATION
OF PROTEIN SYNTHESIS IN CROP PLANTS

\$65,000

Joe L. Key
Department of Botany

The proposed research focuses on the influence of stress agents on protein synthesis in crop plants using primarily soybean. Comparative studies will be directed to analyses of changes in patterns of mRNA synthesis and utilization in tissues subjected to a variety of stress agents (e.g. anaerobiosis, water deficit, salt excess, high temperature, dinitrophenol and supraoptimal concentrations of auxin). These studies should provide new insights into the regulation of protein synthesis by stress agents in plants and to whether some common regulatory mechanism(s) is operative in mediating stress altered protein synthesis by a wide variety of agents.

Investigations into the "heat shock" (stress) mediated changes in transcriptional and translational regulation of protein synthesis in *Drosophila* (see Ashburner and Bonner, 1979) coupled with studies on anaerobic and water deficit mediated alterations in protein synthesis in plants (e.g. Lin and Key, 1967; Hsiao, 1970; Morilla and Boyer, 1973; Freeling, 1973; and Sachs and Freeling, 1978) provide the basis of the proposed research. Approaches will involve analyses of polyribosome state and in vivo rates of protein synthesis, in vitro translation of isolated mRNAs followed by gel electrophoretic fractionation of the proteins, mRNA complexity measurements by cDNA-poly(A)RNA hybridization, cDNA cloning of those poly(A)RNAs engaged in protein synthesis (i.e. ribosome associated) during stress induction (e.g. during anaerobiosis and/or water deficit), and the use of cDNA clones from both normal (clones already available) and the stressed state (e.g. anaerobiosis) to assess the occurrence and distribution of RNAs homologous to those probes in tissues responding to the different stresses and during recovery from those stresses. Of particular importance will be studies on the nature of mRNAs engaged in protein synthesis both during stress induction and recovery from stress. During recovery from stress, patterns of mRNA utilization independent of new RNA synthesis will be emphasized. These studies should provide new insights into (environmental) stress-mediated changes in protein synthesis in plants and possibly provide a base of knowledge important to eventually ameliorating some of the stress effects.

15. UNIVERSITY OF GEORGIA
Athens, Georgia 30602

THE MICROBIOLOGY AND PHYSIOLOGY OF ANAEROBIC FERMENTATIONS OF CELLULOSE \$180,000

Harry D. Peck, Jr. Department of Biochemistry
Lars G. Ljungdahl, " "

The microbiology and physiology of individual microbial types involved in the conversion of cellulose to ethanol, acetate, methane and hydrogen will continued to be studied. Emphasis will be placed on the physiology of the new bacterium, Thermoanaerobacter ethanolicus which produces ethanol by means of a yeast type fermentation at 80°C. Existing programs on the enzymology and bioenergetics of the sulfate reducing bacteria, methanogenic bacteria, and homoacetate fermentors will be continued. We have observed that cellulose is most rapidly fermented to products other than methane when the cellulytic microorganism is grown with a second bacterium capable of fermenting cellobiose. This association will be investigated using the new thermophilic bacterium and Clostridium thermocellum with regard to the parameter responsible for the successful association and the factors effecting product formation. Attempts will be continued to isolate new types of thermophilic and mesophilic bacteria involved in the conversion of cellulose to useful products.

16. HAHNEMANN MEDICAL COLLEGE & HOSPITAL
Philadelphia, Pennsylvania 19102

INVESTIGATION OF THE MOLECULAR MECHANISM OF THERMAL TOLERANCE IN BACILLUS SUBTILIS \$10,000

John J. Ch'ih, Department of Biological Chemistry

In the present work, thermophilic transformants of Bacillus subtilis will be constructed by transformation. B. subtilis will be the recipient, B. caldolyticus will be the donor, and transformation will be carried out by the method of Spizizer according to the procedure described by Lindsay and Creaser. The B. subtilis will be treated with DNA isolated from the thermophile for 4h after which DNase will be added to removed excess DNA. For selecting transformants to grow at high temperature, the treated cells will be allowed to grow at 70° for 18h and then plating for single colonies at 55°. Transformants inheriting streptomycin resistance or adenine prototrophy will be selected. Once a stable thermophilic transformant is isolated, the number of genes responsible for heat stability will be determined and gene will be mapped. If the proposed work is accomplished, subsequent studies will allow us to focus on the precise mechanism of thermal stabilization in determining the actual site of stabilization whether it occurs during transcription or translation. The understanding of the mechanism is of importance to future industrial application such as fermentation process to produce ethanol and other energy-yielding chemicals from biomass.

17. HARVARD UNIVERSITY
Cambridge, Massachusetts 02139

EXPRESSION OF BACTERIAL GENES IN YEAST

\$50,000

Helen Greer, Department of Cellular and
Developmental Biology

We plan to study the expression of prokaryotic genes in the eukaryote yeast, Saccharomyces cerevisiae. Yeast is currently one of the few eukaryotes in which extensive genetic engineering is possible. Procedures for transformation have been developed, along with yeast cloning vectors that carry yeast markers that can be expressed in both E. coli and yeast.

Many genes from gram-negative and gram-positive bacteria have been cloned and are being genetically and physically analyzed. We plan to introduce these specific bacterial genes into yeast via the yeast cloning vectors. This would allow us to determine if bacterial genes can be expressed from their own promoters or if vectors which put cloned segments under a given yeast promoter will be needed for future cloning of bacterial genes in yeast.

An understanding of the expression of prokaryotic genes in yeast is important for several reasons. It will greatly facilitate studies of yeast gene regulation. We will also investigate bacterial transposon functions in yeast. These studies will tell us if it will be possible to clone genes with special functions from bacteria into yeast for industrial applications, specifically for energy sources.

18. UNIVERSITY OF IDAHO
Moscow, Idaho 83843

THE METABOLISM OF AROMATIC COMPOUNDS
BY ACTINOMYCETES

\$67,080

Don L. Crawford, Department of Bacteriology
and Biochemistry

Actinomycetes are a group of filamentous bacteria which are common in nearly all soils. Recent work in my laboratory has shown that certain actinomycetes are able to degrade not only simple hydroxyl- and methoxy-substituted benzoic acids and phenylpropanoid compounds, but also the complex aromatic polymer lignin. Lignin is a major structural component of plants and the second most abundant organic compound on earth next to cellulose. The role of actinomycetes in decomposing this complex aromatic polymer in nature may be of great importance. In the proposed research, we plan to examine the biochemistry of the degradation of low molecular weight aromatic lignin fragments by selected actinomycetes. The principal lignin degradation fragments, released as catabolic intermediates from the lignin polymer as it is decomposed by actinomycetes, will be synthesized and used as substrates for a select group of efficient lignin-decomposing actinomycete strains. The reaction sequences by which these fragments are further degraded through single-ring aromatic intermediates to final products will be elucidated. In particular, groups of genetic mutants blocked at various points in the metabolism of specific fragments will be utilized to accumulate catabolic intermediates in isolatable amounts. Success in this research may result in the possible development of bioconversion processes for converting lignin fragments into basic and valuable phenolic chemicals. Thus, lignin might serve as a renewable source of many of the vital chemical feedstocks now derived from our dwindling supply of nonrenewable petroleum.

19. UNIVERSITY OF ILLINOIS
Urbana, Illinois 61801

PHOTOSYNTHESIS IN INTACT PLANTS

\$96,500

A. R. Crofts

Department of Physiology and Biophysics

Photosynthesis in the field as measured by yield normally occurs at rates 5-10 fold lower than those measured under optimal field conditions. It is not known how environmental factors affect the net photosynthetic yield, but for some forms of stress, it is known that the photosynthetic mechanism is directly inhibited at the level of the electron transfer chain. Investigation of such factors is greatly restricted by limitations in the methodology available for studying intact plants. The application proposes a program to develop instrumentation and expertise for the investigation of photosynthetic mechanisms in intact plants. The apparatus to be constructed will be based on biophysical techniques already shown to be effective in the study of isolated chloroplasts and algae, but will include novel features to permit the development of portable field equipment. The program proposes a number of research projects of general interest in photosynthesis, and suggests areas of investigation of immediate relevance to agricultural productivity and the generation of biomass.

The program has three main elements:

- a) Construction of instrumentation for measuring the rapid kinetics of changes in absorbance or of fluorescence yield, and for measuring induction of fluorescence and delayed fluorescence; it is intended that the instruments will be developed in a form suitable for laboratory or field use.
- b) Laboratory based research, using intact leaves, on the mechanism of herbicide action, and of herbicide tolerance; the role of cyclic photosynthetic electron transport in intact leaves; the kinetics and redox poise of the photosynthetic electron transport chain; the effects of water deficiency and heat stress on electron transport.
- c) Research in the field, in which our laboratory experience will be used to investigate the effects of environment on photosynthetic capacity at the level of the reactions of electron transport and energy conversion. This latter phase of research will involve extensive collaboration with personnel of the School of Agriculture, and the USDA group on campus.

20. UNIVERSITY OF ILLINOIS
Urbana, Illinois 61801

BIOCHEMICAL AND BIOPHYSICAL STUDIES ON THE
E. COLI AEROBIC RESPIRATORY CHAIN

\$58,200

Robert B. Gennis
Department of Chemistry & Biochemistry

The long term goal of the proposed research is to understand at a molecular level both the structure and the function of the E. coli aerobic respiratory chain. This chain consists of a series of electron and proton carrying components within the cytoplasmic membrane of the organism. On the basis of what is already known, it is expected that this bacterial electron transport chain will be considerably simpler in terms of the number of components than the corresponding electron transport chain of the mitochondrion. There are two major motivations behind this project. The first is to obtain an understanding at the molecular level of the mechanisms of electron transport and of energy conservation. In particular, we wish to address the question of how electron transport is associated with the generation of an electrochemical transmembrane potential. Secondly, this system offers the opportunity to study a functional membrane-bound multiprotein network. The role of protein-lipid interactions and of protein-protein associations in such networks is important to understand if we are to comprehend how membranes are structured and function.

A major portion of the proposed work will be to purify and characterize one of the two terminal oxidases found in E. coli. The mechanism by which oxygen is reduced will be studied in addition to the manner in which this enzyme is embedded in the bacterial membrane. A combination of biochemical, immunological, and genetics techniques will be used to identify, isolate and characterize other components of the membrane required for electron transport. Crossed immunoelectrophoresis will be used extensively in this project. The structure of the electron transport chain as well as the mechanism of proton pumping and energy conservation will eventually be studied by the use of reconstitution procedures.

21. UNIVERSITY OF ILLINOIS
Urbana, Illinois 61801

ACETOPHILIC METHANOGENIC CONSORTIA
Ralph S. Wolfe
Department of Microbiology

\$80,000

We propose to study the development and subsequent analysis of acetophilic methanogenic consortia that are developed by use of natural as well as synthetic feedstocks in the laboratory. Since acetophilic methanogens are responsible for about 70% of the methane produced in anaerobic digestion, it is important to thoroughly understand these organisms. Only one species is currently in pure culture, and we propose to make a detailed study of its physiology, nutrition, interaction with other anaerobes or facultative anaerobes, and methanogenesis in the absence of growth. We propose to isolate new acetophilic methanogens seeking answers to questions such as: Why do some of these organisms have a generation time of 9 to 15 days or more? Why does it take 3 months or more to obtain a colony large enough to study? What are the factors that allow these organisms to grow better in mixed cultures? Why are they so difficult to isolate? We propose to study the assembly of methanogenic consortia from pure cultures that we isolate from active consortia, defining the factors that are involved in the physical association and in the physiological interaction of the microbial components.

22. IOWA STATE UNIVERSITY
Ames, Iowa 50011

POST-ILLUMINATION CO₂ EVOLUTION AND
PHOTORESPIRATORY METABOLITE LEVELS

\$69,244
(24 months)

Cecil R. Stewart

Department of Botany and Plant Pathology

The purpose of this project is to develop a valid measure of photorespiration in plants which, in many cases, represents a loss of 25% to 30% potential biomass production. The post-illumination CO₂ evolution (PIO) will be measured and mathematical correction for nonsteady state conditions applied to the measurements on leaves of soybean and other C₃ plants. A measuring system will be developed that gives corrected rates that are independent of flow rate and other geometric characteristics of the system. Photorespiratory intermediary metabolite levels will be measured and the post-illumination disappearance of ribulose-1,5-bisphosphate (RuBP), glycolate and glycine will be monitored. The rate of disappearance of glycolate plus glycine in the time interval immediately following the disappearance of RuBP will be compared with the post-illumination CO₂ evolution to validate the PIO as a measure of photorespiration. These rate measurements will be made and compared under varying O₂ and CO₂ levels. If the rates compare favorably under different conditions and in different species, it will constitute evidence that the corrected PIO is a valid measure of photorespiration.

If this method is valid, it will be useful to plant scientists interested in increasing productivity by regulating photorespiration. It would be specifically useful in screening compounds that limit photorespiration and genotypes of plants that are important or potentially important in biomass production.

23. MARTIN MARIETTA LABORATORIES
Baltimore, Maryland 21227

PHOTOCHEMISTRY AND ENZYMOLOGY OF PHOTOSYNTHESIS \$80,000
Richard J. Radmer
John Golbeck
Bruno Velthuys
Biosciences Department

This project is part of an extensive photosynthesis research program that has the ultimate purpose of seeking a means of increasing photosynthetic productivity. The project addresses three different aspects of photosynthesis and uses three (for the most part) different technical approaches (i.e. mass spectrometry; biochemical techniques; and a combination of polarographic, spectroscopic, and fluorescence techniques). In the mass spectrometer experiments we will analyze the products (and their isotopic composition) evolved as a result of single turnovers of the photosynthetic apparatus in the presence of H₂O or artificial donors. These studies should help to elucidate reactions occurring during the water splitting (O₂-evolving) process. The biochemical studies will be directed toward establishing the identity of copper-containing chloroplast components (other than plastocyanin). These studies should help to clarify several aspects of the photosynthetic process, e.g. whether or not a copper-containing protein functions in the O₂-evolving process. The third part of this project is directed toward studies on the distribution of photons, reducing power, and ATP in the mesophyll and bundle sheath tissue of C₄ plants. These studies should help to clarify the mechanisms by which plants of this group are often able to attain very high growth rates and efficiencies.

24. STATE UNIVERSITY OF NEW YORK
Binghamton, New York 13901

GENE-ENZYME RELATIONSHIPS IN SOMATIC CELLS AND \$75,000
THEIR ORGANISMAL DERIVATIVES IN HIGHER PLANTS
Roy A. Jensen
Department of Biological Sciences

Elucidation of the regulation of biochemical functions in eukaryotic cells is of paramount importance in understanding tissue differentiation, organismal development, and abnormal instances of differentiation. The use of mutations to elucidate the control of metabolic functions is well established in prokaryotic organisms. The feasibility of managing cultured plant cells in the haploid state allows the exploitation of microbiological approaches with eukaryotic cells which are capable of regeneration to the organismal state. This study is intended to achieve insight into the control of biochemical functions and the relationship of this control to tissue differentiation and organismal growth.

25. UNIVERSITY OF NORTH CAROLINA
Chapel Hill, North Carolina 27514

THE ISOLATION AND CHARACTERIZATION OF
B-GLUCOSIDASE GENE AND B-GLUCOSIDASE
OF TRICHODERMA VIRIDE

\$80,000

Darrel W. Stafford, Department of Zoology
Roger L. Lundblad, Dental Research Center

We propose to begin studies which will eventually lead to an organism with the following properties: the organism should carry a multiple copy plasmid with all the necessary genes for cellulase production under the control of one strong promoter; the genes should carry information for the "leader" protein sequence which is necessary for the proteins to pass through the cell membrane into the environment; the genes themselves should have been mutated in vitro to eliminate end-product inhibition; and, finally, the genes should be inserted into a yeast host so that the glucose produced from cellulose could be converted directly to ethanol. An alternative, also very attractive, would be to reinsert the cloned, modified genes back into Trichoderma viride together with genes from yeast to allow the complete conversion of cellulose to ethanol by Trichoderma.

The result of the specific research we are proposing for the 3-year period of this proposal will be (a) clones of E. coli that contain recombinant DNA including the gene for B-glucosidase in fragments of DNA originally purified from Trichoderma, and (b) clones of E. coli that contain recombinant DNA including copy DNA synthesized via reverse transcriptase from the messenger RNA that codes the sequence of amino acids in B-glucosidase. The Trichoderma DNA fragments can be recovered from the clones, and the sequence of their nucleotides determined and compared to characterize the nucleotide sequence associated with B-glucosidase production and function. During this early phase of our research, we will also determine the amino acid sequence of B-glucosidase and try to locate that portion of the sequence responsible for the inhibition of B-glucosidase activity by the end-product, glucose.

26. UNIVERSITY OF OKLAHOMA
Norman, Oklahoma 73069

DETERMINE IF INFLUENCE OF SINK DEMAND ON NITROGEN \$50,000
METABOLISM IN THE SOURCE LEAF CAN ACCOUNT FOR
REGULATION OF PHOTOSYNTHETIC ACTIVITY
John S. Fletcher, Department of Botany-Microbiology
Leonard Beevers, " " "

The rate of photosynthesis is one of the important factors affecting plant growth. It is now recognized that growth itself can affect the photosynthetic rate, and instead of there being a one-way relationship, source-photosynthesis \rightarrow sink-growth, there is an interrelationship, source-photosynthesis \leftrightarrow sink-growth. Thus, there is an internal regulation between sink and source.

It has been suggested that during reduced sink demand assimilates accumulate in the leaf and inhibit photosynthesis. However, the assimilates involved and their mode of action have not been identified. Research directed primarily towards relating carbohydrate accumulation to a decline in photosynthesis has failed to show a clear relationship. No attention has been given to the possibility that nitrogenous compounds may regulate photosynthetic activity of the source.

The present study aims at demonstrating the influence of reduced sink demand on light dependent nitrate assimilation and metabolism of nitrogenous compounds. Fluctuations in nitrogenous compounds will be related to changes in photosynthetic rates with a view of establishing the chemical basis for sink control. Nitrogen-15 will be used to follow nitrate assimilation and photosynthetic rates will be measured with an infra-red analyzer.

27. UNIVERSITY OF PENNSYLVANIA
Philadelphia, Pennsylvania 19104

FACTORS GOVERNING LIGHT DRIVEN ELECTRON AND PROTON TRANSLOCATION IN PROTEINS ACROSS MEMBRANES \$75,000
P. Leslie Dutton
Department of Biochemistry & Biophysics

We propose to study photochemical reaction centers from the photosynthetic bacterium Rhodospseudomonas sphaeroides to better understand the factors that govern the process of light-activated electron transfer and charge separation effected by redox components that are held within a protein matrix and directed across a membrane.

Central to many experiments will be reaction centers modified by the replacement of the primary (and secondary) ubiquinone-10 with other redox chemical structures. This will provide the opportunity to systematically and widely vary the free energy available between the reacting couples of the reaction center. Secondly, it will permit us to develop reaction centers with a free energy arrangement that is closer to the energetic limit of proper function than is evident in the natural system; this should yield an experimentally more responsive system. Thirdly, ubiquinone-replaced reaction centers will be used to probe the binding environment of the ubiquinone. Finally ubiquinone-replaced reaction centers will be developed that will allow us to perform physical-structural studies on the reaction center that otherwise would not be feasible.

Reaction centers, normal and modified, will be studied in three environments: in the natural membrane, in the detergent isolated state, and after reincorporation into phospholipid bilayers. Each environmental state offers its own experimental advantages.

The effect of modification will be examined by determination of flash-induced kinetics, activation energy, quantum efficiency and membrane charging capability. In the planar bilayers, both current- and voltage-clamped techniques will be applied; the reaction center will be studied in combination with membrane impermeable electron donors on one side and electron acceptors on the other side of the membrane, as well as with lipid soluble redox (hydrogen) carriers to make a cyclic system. We shall also endeavor to achieve a higher level of sophistication in this regard by combining the reaction center in the planar membrane with its sister protein, the ubiquinone-cytochrome b/c₂ oxido-reductase; these two proteins in the natural membrane form a light driven, cyclic electron transfer system that pumps H^+ ($2H^+/e^-$) across the membrane without need for net input of oxidants or reductants.

This program is designed in the hope that it will reveal new facts pertaining to the operation of these highly organized energy transducing proteins. It is anticipated that this information will be of importance to current theories and hypotheses on the mechanisms of electron and ion translocation. At the same time we hope to obtain some guidelines that will ultimately be of practical value in the construction of submicron devices relevant to this topic.

28. PURDUE UNIVERSITY
West Lafayette, Indiana 47907

SEED PROTEIN GENES AND THE REGULATION
OF THEIR EXPRESSION

\$132,938
(14 months)

Brian A. Larkins, Department of Botany
and Plant Pathology

Donald E. Foard, Department of Botany
and Plant Pathology

The proteins of seeds provide useful model systems for studying gene regulation in plants. This proposal presents experiments to study the zein genes of maize, and the low molecular weight protease inhibitor (LMWPI) genes of soybean. Zein genes code for the major storage proteins in maize endosperm. A good deal of information is now available regarding the nature of zein proteins, their mRNAs, and the mechanism of synthesis and deposition of these proteins in the endosperm. The LMWPIs of soybean seeds are a group of well characterized proteins that account for approximately 50% of the total sulfur-containing amino acids in the soybean seed. Although these proteins are structurally well characterized, little is known about the genes coding for them, nor the regulation of their biosynthesis. We propose to analyze the transcription of zein genes in Xenopus laevis oocytes. Experiments will be done to determine the fidelity and specificity of their transcription, as well as the relationship between gene structure and transcriptional regulation. Experiments are also presented to prepare enriched fractions of LMWPI mRNAs. These mRNAs will subsequently be cloned in bacterial plasmids using recombinant DNA techniques. Clones containing cDNAs corresponding to LMWPIs will be isolated from among the recombinants, and they will eventually be used to study the structure and expression of LMWPI genes.

29. ROCKEFELLER UNIVERSITY
New York, New York 10021

DIFFERENTIAL GENE EXPRESSION IN C4 PLANTS

\$80,000

Anthony R. Cashmore
Nam-Hai Chua

A major product, if not the major product, of plant nuclear gene expression is the small subunit of ribulose-1,5-bisphosphate carboxylase. A well defined example of differential expression of the gene encoding this polypeptide is found in the mesophyll and bundle sheath cells in C₄ plants. The small subunit is a major polypeptide in bundle sheath cells, but it is not detectable in mesophyll cells. We intend to examine the factors determining the differential expression of these genes in the mesophyll and bundle sheath cells of maize. A primary aim of this project is to determine whether the point of control is at the level of translation, post-transcription or transcription. Preliminary results suggest that control is exerted at the transcriptional or post-transcriptional level. We will attempt to characterize, for the first time, plant precursor mRNAs, in view of the possibility that control of nuclear gene expression may be regulated by the processing and transport of these RNAs. In addition, we will examine by molecular hybridization, the possibility that DNA sequence rearrangements are involved in the differential expression of these genes. This project should make a significant contribution to our understanding of the regulation of plant nuclear genes.

30. RUTGERS UNIVERSITY
Piscataway & New Brunswick, New Jersey 08854

SECRETORY MECHANISMS OF MICROORGANISMS: CELLULASE \$70,625
SECRETION BY TRICHODERMA REESEI

Bijan K. Ghosh, Department of Physiology and
Biophysics - Piscataway
Bland S. Montenecourt, Department of Biochemistry
and Microbiology - New Brunswick
Douglas E. Eveleigh, Department of Biochemistry and
Microbiology - New Brunswick

Cellulosic biomass can be developed as a renewable resource to supply 10% of the annual U. S. needs. This abundant material is recycled through the action of microbial enzymes, cellulase. However, in order to reach and attack the insoluble cellulose these enzymes must be secreted from the cell. The economy of an industrial conversion process will partly depend upon the production cost of the enzyme. Increased synthesis of cellulase per unit cell mass will result in reduction of the cost of enzyme production. Thus, the ultimate aim of this investigation is to increase the capability of the cells (by some simple means) both to synthesize and to secrete cellulase. Considerable headway has been made to increase the cellulase synthetic capacity of the cells through genetic engineering. However, the increased ability of the cells to synthesize an enzyme does not necessarily imply the concomitant greater ability of the cells to transport the enzyme outside the cell body, as synthesis and secretion are regulated by independent control mechanisms. The lack of knowledge of the physiological mechanism and regulation of enzyme secretion in fungi is a stumbling block in using a simple factor to stimulate the physiological process of secretion, or to use genetic engineering to increase the secretability of the cells.

The aim of this investigation is first, to understand the mechanism of cellulase secretion in Trichoderma reesei and second, to identify a step of the secretory process which can be modified by a simple exogenous factor or by genetic engineering which will result in increased secretion. This investigation focusses on cellulase, an enzyme critical in the utilization of biomass. This study will allow development of a microbial system in which a rapid and effective enzymatic saccharification of cellulose will be achieved. It will also allow development of more efficient processes that can be applied to other microbial systems.

31. SMITHSONIAN INSTITUTION
Rockville, Maryland 20852

A PRIMARY LIGHT HARVESTING SYSTEM: PHYCOBILISOMES \$30,000
AND ASSOCIATED MEMBRANES
Elisabeth Gantt
Radiation Biology Laboratory

In red and blue-green algae phycobilisomes, composed of phycobiliproteins, act as energy sinks funnelling the energy to chlorophyll in the photosynthetic membranes with an efficiency of up to 95%. The objectives are:

- 1) To demonstrate the mechanisms of energy transfer occurring between phycobilisomes and photosynthetic reaction centers;
- 2) To determine the specific binding of phycobiliproteins within phycobilisomes;
- 3) To identify and isolate the binding components between the photosynthetic membranes and phycobilisomes.

Purified allophycocyanins, the terminal pigment forms in the transfer chain are being characterized by fluorescence, absorption, and circular dichroism at 23° and -196°C. With isolated heteromere complexes, composed principally of phycoerythrin and phycocyanin, in vitro dissociation and recombination dynamics are explored with varying ionic conditions, temperature and pressures up to 40,000 psi. Fluorescence and Hill activity assays are used to assess energy transfer capabilities in vitro from phycobilisomes to photosynthetic membranes.

32. SMITHSONIAN INSTITUTION
Rockville, Maryland 20852

SOLAR ENERGY SPECTRAL QUALITY MEASUREMENTS \$67,000
AS RELATED TO GROWTH AND DEVELOPMENT OF PLANTS
William H. Klein
Radiation Biology Laboratory

Objectives: (A) the measurement of daily and seasonal fluctuations in spectral distribution of total sky irradiance, (B) its control of plant growth and development and (C) the effects of pulsed light on plant productivity and metabolic efficiency are currently being investigated. Approaches: (A) an integrating scanning radiometer has been designed to continuously monitor both the UV-A (320-390 nm), UV-B (285-320 nm), and visible (400-800 nm) irradiation at 3 sites: Barrow, Alaska (71°N); Rockville, Maryland (39°N); and Panama City, Panama (9°N). (B) The effect of far-red (700-800 nm) supplementing daylight fluorescent light on the induction of flowering in barley relative to continuous light. Results: (A) Develop, construct, test, and place in operation integrating scanning radiometers for accurate, reliable monitoring of the solar irradiances in the three spectral bands and operate this monitoring system to provide a data base for use in photobiology. (B) Determine the photoperiodic response and the control mechanism for flowering activated by spectral quality and irradiance changes found in the data at twilight. (C) Evaluate the effects of pulsed light on photosynthesis and light distribution relative to continuous light.

33. SRI INTERNATIONAL
Menlo Park, California 94025

REGULATORY MECHANISMS IN THIELAVIA TERRESTRIS

\$49,999

Daniel Tusé
Life Sciences Division

SRI International has isolated a strain of the thermophilic fungus Thielavia terrestris. This organism produces a heat-stable cellulase that can degrade both amorphous and crystalline cellulose to glucose; hence it may be of interest for converting cellulose into fuels. Reports in the literature and our own findings suggest that Thielavia is also hemicellulolytic and weakly lignolytic. We wish to study the regulatory mechanisms used by this fungus to sense and attack insoluble extracellular polymers. We propose to study, at the molecular level, the physiology and genetics of the induction processes which lead to the synthesis and release of lytic enzymes that depolymerize substrates such as cellulose, hemicellulose, and lignin.

We will concentrate our research efforts on determining the relationships between induction of lytic enzymes and (1) the necessity of cell-polymer contact, (2) the nature of the inducer, (3) the role played by enzyme activity, and (4) the location of the genetic information that codes for lytic enzyme synthesis in Thielavia terrestris. The information we generate will further our understanding of fungal regulatory schemes, extracellular sensing mechanisms, and the physiology and genetics of the interactions between eukaryotes and polymers, and will be a valuable prelude to possible recombinant DNA work.

34. UNIVERSITY OF WASHINGTON
Seattle, Washington 98195

STUDIES ON THE CONTROL OF PLANT CELL ENLARGEMENT BY CELLULAR PARAMETERS \$40,554
Robert E. Cleland
Botany Department

The size of plants, and thus the amount of biomass available for use as food and energy, depends upon the extent to which individual cells enlarge. Cell enlargement is controlled by five factors; the hydraulic conductivity, the osmotic potential, the wall yield stress, the rate of proton excretion, and the wall loosening capacity. When a cell is induced to enlarge, one or more of these five factors must change so as to permit the expansion. The goal of this research is to measure each of these factors, for the first time, for a single system; the auxin-induced expansion of oat coleoptile cells. During the current year we are concentrating on the changes which occur in the osmotic potential and in the wall loosening capacity.

35. UNIVERSITY OF WASHINGTON
Seattle, Washington 98195

A STUDY OF THE GENETICS AND REGULATION OF METHANE OXIDATION \$51,385
Mary L. O'Connor
Department of Microbiology and Immunology

The purpose of this project is to identify the genes involved in methane oxidation and to determine how they are regulated.

In order to accomplish this goal, the methane mono-oxygenase will be purified from a facultative methanotroph and characterized. Once the components of this enzyme are identified, mutants deficient in methane and methanol oxidation will be isolated and characterized. A genetic system will then be developed, and the mutants which have been isolated will be mapped against themselves and against marker mutants. The lesions will be localized in the chromosome or on the methane plasmid.

These experiments will provide information which will be used to determine the number and location of genes involved in methane oxidation. In addition, some insight into the overall regulation of methane oxidation will be gained.

36. UNIVERSITY OF WISCONSIN
Madison, Wisconsin 53706

DEVELOPMENT OF NITROGEN-FIXING MONOCOT-
BACTERIA ASSOCIATIONS

\$70,000

Winston J. Brill
Department of Bacteriology

We have developed an assay for corn-root material capable of supporting N_2 fixation by Azotobacter. With this assay, which we call the association assay, we have screened about 1600 young corn plants grown from seeds from a wide variety of sources. Although the great majority of plants were not capable of supporting N_2 fixation by Azotobacter, we did find a few active plants. We bred these plants and found that activity by the association assay is dependent on the genetics of the plant. We can breed activity into adapted inactive lines. This suggests that the genetic components tend to be dominant and should be easy to manipulate in further breeding.

We propose to continue our screening and breeding program to increase N_2 fixation in the association assay. We will determine which bacterial strains are most active with active plants. We will select bacterial mutants that utilize photosynthate efficiently, and we will test ammonium-excreting mutants for improved N fertilization of corn. We plan to use N isotopes and Kjeldahl N increases to determine N fertilization of plants and we will work toward stabilizing promising associations in the field. Field trials will determine if the associations we develop have agronomic value.

Our goal is applied, but we will also contribute to important basic concepts. We expect to increase understanding of the roles of root exudates, mucilage, or sloughed off cells in N_2 fixation. We will gain insights into the dynamics of rhizosphere ecology. We may learn valuable information about the genetics of the formation of root material able to support N_2 fixation. The methodologies we develop for corn, and the basic scientific advances we expect to make with this project, may be applicable to other plants. As a result of the work we propose, it may be easier to develop N_2 -fixing associations with other important crops.

37. UNIVERSITY OF WISCONSIN
Madison, Wisconsin 53706

ORGANIZATION OF THE R CHROMOSOME REGION IN MAIZE \$32,400
Jerry L. Kermicle
Laboratory of Genetics

The R chromosome region in maize is under study with a view to determining the number, kind, and arrangement of the components involved in the control of anthocyanin pigmentation. Organization of the region is investigated by analysis of the variation found within land races or that arising in experimental cultures. Changes arise in culture spontaneously by mutation or intra-locus recombination, are induced by chemical mutagens, and occur in appropriate heterozygotes through paramutation. The classification of intra-locus recombinants for outside markers permits recognition and serial ordering of the various R components. Characterization for simultaneous changes in less conventional properties, such as paramutation, permits the corresponding determinants to be ordered spatially relative to the pigmenting elements. Particular attention is being given the nature of tissue-specific R expression and a cyclic pattern of variation characteristic of certain alleles.

38. UNIVERSITY OF WISCONSIN
Madison, Wisconsin 53706

ONE CARBON METABOLISM IN ANAEROBIC BACTERIA: \$65,000
ORGANIC ACID AND METHANE PRODUCTION
J. G. Zeikus
Department of Bacteriology

Acetate, butyrate and methane are important sources of industrial chemicals or fuel that are produced as a result of the bacterial fermentation of one carbon compounds (e.g., methanol, carbon monoxide or H_2/CO_2). These substrates are readily available as pyrolysis products of biomass or coal. This research aims to understand the pathways and regulation of one carbon metabolism in acidogenic and methanogenic bacteria by detailed physiological, biochemical and genetic studies. These investigations will characterize and compare methanol, H_2/CO_2 , CO and acetate metabolism of Methanosarcina barkeri and a new acidogenic species, the Marburg strain. The results have potential process applications for the production of C_2 and C_4 chemical feedstocks, vitamins and methane from renewable resources or coals.

BROOKHAVEN NATIONAL LABORATORY
Department of Biology
Upton, New York 11973

Total Funding: \$790,000

39. PLANT PHYSIOLOGY 2.6
William S. Hillman

The biological effects of intermittent environmental conditions, whether considered as stresses (e.g., pollution or extreme temperatures) or as normal (e.g., daily light and darkness) depend strongly on timing. Exposures a few hours apart may have drastically different outcomes. This situation reflects the interaction of biological timing processes and external signals, chiefly light, that can reset them, and is crucial in evaluating the action of many environmental factors.

With the long-term goal of understanding and modifying time-dependent environmental responses, the regulation of development by the daily light schedule, known as photoperiodism, is being studied as a model. Photoperiodism is also important in itself as a control in the reproduction of both plants and animals. The immediate approach involves analysis of the daily respiratory circumstances, these daily respiratory patterns respond to differing light schedules in the same manner as does the photoperiodic control of flowering. The experiments also bear on the regulation of respiration by low-intensity light, and thus on the energy effectiveness of plant productivity.

40. PLANT CELL GENETICS 2.5
Benjamin Burr
Frances Burr

It is important to find ways of making green plants more efficient users of solar energy in the production of biomass and of protein for human consumption. Past advances in this area have come from plant breeding. Recent advances in molecular genetics suggest radical ways to adapt plants to human use but these techniques await development. One of the problems to be overcome is learning how to introduce foreign genes, conferring beneficial properties, into crop species. The use of small, independently-replicating units of DNA known to exist in corn as potential carriers of genetic material is being explored. The description and definition of existing genetic entities, known to transpose within the corn genome, is also being attempted. The transposable elements may aid in the mobilization of genes for transfer and in their integration into a recipient genome. Techniques to be employed include: 1) molecular cloning of complementary DNA made from partially purified messenger RNA, or cloning of sequences isolated directly from the corn genome; 2) determination of the structure of normal genes and transposable elements by the use of restriction enzymes and nucleic acid sequencing techniques, and 3) attempts to repair mutant genotypes with introduced recombinant DNA.

Biology Department, BROOKHAVEN NATIONAL LABORATORY, continued

41. PHOTOSYNTHETIC MEMBRANES

2.0

John M. Olson

When the structure and function of photosynthetic membranes are understood in sufficient detail, it may prove possible to construct solar energy converters based on biological principles and able to transform sunlight into chemical or electrical energy with an efficiency comparable to that of silicon cells. In photosynthetic membranes, light absorbed by antenna chlorophyll is converted to excitation energy which is transferred to photochemical reaction centers. (The primary photochemical reaction is an electron transfer from an excited chlorophyll dimer to an acceptor molecule, either a quinone or an iron-sulfur center. This primary reaction drives photosynthetic electron transfer in a system of cytochromes, quinones, iron-sulfur proteins, and other components.) The efficiency of energy trapping depends on the spatial organization of antenna chlorophyll in relation to reaction centers. Structural relationships between bacteriochlorophyll a (Bchl a)-proteins and reaction centers are being studied in unit-membrane vesicles from green bacteria, because the structure of the Bchl a-protein is known to a resolution of 0.28 nm and can in principle be identified and localized within the unit membrane. Bchl a-proteins have already been isolated and characterized, and currently lipids are being investigated. Eventually reaction centers will also be isolated and studied. When the interactions between these membrane components are elucidated, the resulting structural picture of the membrane should explain its function in the primary steps of energy conversion.

Biology Department, BROOKHAVEN NATIONAL LABORATORY, continued

42. MECHANISMS OF ENERGY CONVERSION IN PLANTS
Geoffrey Hind

4.0

In C-3 photosynthesis, cyclic electron transport around photosystem I has been shown to provide some of the ATP needed to fix CO₂; this project continues to expand knowledge of the electron carriers so involved. Cyclic electron flow is thought to be even more active in the bundle sheath chloroplasts of plants having the C-4 photosynthetic carbon pathway and a consequently higher requirement for ATP relative to reductant (NADPH). Cyclic generation of ATP by photosystem I is also considered essential for the reduction of nitrogen in heterocysts of filamentous blue-green algae. Isolation techniques for the aforementioned photosynthetic membrane systems have been or will be developed to permit study of their electron transport and energy coupling mechanisms.

The special conditions needed during chloroplast isolation to retain intactness and cyclic electron flow, seem also to facilitate subsequent light activation of the thylakoid ATPase (CF₁). This possible direct link between electron flow and ATP turnover is under study in spinach chloroplasts. The vacuole membrane of plant cells contains a coupling factor resembling that of chloroplasts and the vacuole system offers technical advantages which recommend its use for some phases of this investigation.

Biology Department, BROOKHAVEN NATIONAL LABORATORY, continued

43. PLANT CELL GENETICS 1.0
Harold H. Smith

The overall objective of this project is to gain an understanding of genetic controls in plant development through the use of cell and tissue culture and the analysis of genetic tumors. Various enzymatic techniques provide protoplasts, that is, wall-less cells, for fusion and parasexual hybridization, making possible expanded studies of somatic cell genetics. Interspecific hybridization has been accomplished in this way and the methods of fusing somatic protoplasts are being extended to intergeneric hybridization and even to interkingdom fusion. These new methods for combining more widely divergent genomes than previously possible may find use in the production of entirely new kinds of living organisms. Haploid cell lines are established by anther culture and these cultures are selected for resistance to analogs and antimetabolites, then analyzed to determine the causes for the resistance. These new methods are expected to find use in the production, more rapidly than previously possible, of plants resistant to various pollutants, thus permitting biological productivity to be maintained.

The genetic basis of spontaneous tumor formation in plants is studied by isolating particular chromosomes that, when introduced into another species cause tumors to develop. These chromosomally-defined tumor-prone plants are then grown in tissue culture to determine their differences from normal plants in physiological or biochemical growth factor requirements. The experiments demonstrate the importance of a genetic component in causing tumors.

Biology Department, BROOKHAVEN NATIONAL LABORATORY, continued

44. STUDIES BEARING ON THE USE OF LEMNACEAE (DUCKWEEDS) 1.5
AS BIOMASS/NATURAL PRODUCT CROPS AND FOR WATER
POLLUTION CONTROL
William S. Hillman

Dependence on fossil energy sources could be decreased by the use of rapidly-growing organisms that do not compete with traditional agriculture for space and nutrients and that nevertheless can serve as biomass, as natural-product sources, or as animal feed. Aquatic plants able to grow in polluted conditions--for instance, in nutrient-rich effluents from energy-intensive agriculture and from food processing--or in shallow ponds of high salinity would be especially valuable.

The Lemnaceae--duckweeds--utilize both solar energy, through photosynthesis, and fixed energy sources, in the form of dissolved organic compounds. They grow more rapidly than other higher plants due to their floating habit and small but macroscopic size. Their habit and size also make harvest far easier than that of the micro-organisms, including unicellular algae, often proposed as biomass crops and for treating polluted water. Hence the purpose of this program is two-fold; to study several physiological processes in the Lemnaceae, such as nitrate nutrition, that are relevant to their use as indicated; and to carry out at least preliminary attempts to produce strains adapted to growth under adverse (high salinity) conditions.

Biology Department, BROOKHAVEN NATIONAL LABORATORY, continued

45. PHOTODESALINATION RESEARCH AND DEVELOPMENT
Geoffrey Hind

2.0

This project aims to develop a mechanically stable membrane that will use solar energy to pump salts from sea or saline water to the point of desalination (< 500 ppm NaCl). Pigment complexes from biological sources will be oriented in the membrane to drive proton fluxes against the concentration gradient, at the expense of absorbed light energy. Ionophores will be incorporated into the proton-pumping membranes to give fluxes of Na^+ and Cl^- . Two types of proton pump, having complementary light absorbance, are under consideration as the primary energy transducers:

(1) studies with isolated thylakoids have shown that electron transport and charge separation are vectored so that an electrical field develops across the membrane and protons flow inwards in the light. This process can accompany cyclic electron flow around photosystem I through a chain of incompletely identified electron carriers. Particles containing only photosystem I and some of the known carriers can be isolated; when reconstituted and oriented on a membrane support, they may serve as a vectorial proton pump.

(2) proton gradients can be directly generated upon illumination of the purple membrane from Halobacterium species, after its orientation in liposomes or on planar synthetic supports.

Biology Department, BROOKHAVEN NATIONAL LABORATORY, continued

46. GENETIC ENGINEERING IN PLANTS 2.5

Daniela Sciaky

Crown gall is a naturally occurring genetic engineering system where bacteria transfer and express their genes in plants. The components of crown gall responsible for transfer and integration of the T-DNA in plants will be isolated and used to construct a plant cloning vector using the now conventional molecular cloning techniques. This will require: 1) the construction of a cloning vehicle capable of replication in the bacterial host (Agrobacterium tumefaciens) and integration into the plant DNA; and 2) the characterization of the bacterial DNA responsible for insertion and expression in the plant host so that insertion of foreign genes into the cloning vector will not inactivate the system or not allow the foreign genes to be expressed.

In the course of construction of this vector or transfer plasmid the boundaries of the T-DNA on the bacterial plasmid responsible for tumorigenesis (the Ti plasmid) will be delineated. Other genes involved in the final expression of T-DNA will be mapped and their ability to trans complement the T-DNA will be determined.

Plant RNA homologous to the T-DNA will be characterized by restriction digests and sequencing to determine whether these messages are spliced. If so, this will be the first case of plant nuclear genes that are transcribed as spliced messages.

LAWRENCE BERKELEY LABORATORY
Chemical Biodynamics Division and Donner Laboratory
Berkeley, California 94720

Total Funding: \$675,000

47. ENERGY CONVERSION IN PHOTOSYNTHESIS 2.5
Kenneth Sauer, Chemical Biodynamics Division
Melvin P. Klein, " "

Photosynthetic membranes where energy conversion occurs contain chlorophyll and other pigments, electron transport cofactors and sites of enzymatic coupling in an organized arrangement of proteins in a lipid matrix. We are investigating the pattern of this organization at the molecular level using optical and magnetic resonance techniques applied to oriented samples and polarized photoselection applied to unoriented samples. We have determined the orientation of triplet species formed by radical pair recombination in the reaction centers of photosynthetic bacteria.

Following the absorption of light in the antenna pigments in the membranes, the resulting electronic excitation migrates over hundreds of chlorophyll molecules within a few picoseconds before it reaches the reaction centers. There the excitation induces the separation of an electron and a hole within about 1.5 ps. Using optical and EPR techniques we are able to follow the path of the electron as it moves from one acceptor to the next. Our discovery of electron spin polarization in the reaction centers has led to an interpretation of the charge separation in terms of a Radical Pair Mechanism. Development of a detailed theoretical formulation of the origin of spin polarization has allowed us to put together a reasonable mechanism for the primary energy conversion steps in photosynthesis.

The oxidation of water to molecular oxygen is an important aspect of higher plant photosynthesis. Manganese is implicated as an intermediate in this process, and we have uncovered the first direct evidence for its participation in the redox reactions in water splitting. Structural and kinetic studies show that two Mn atoms cooperate in storing oxidizing equivalents in the course of removing 4 electrons from two water molecules to produce molecular oxygen.

Chemical Biodynamics Division, LAWRENCE BERKELEY LABORATORY

| | | |
|-----|---|-----|
| 48. | PLANT BIOCHEMISTRY | 4.0 |
| | James A. Bassham, Chemical Biodynamics Division | |
| | Henry Rapoport, " | " |
| | Melvin Calvin, " | " |

The objectives of this task are to determine photosynthetic and biosynthetic pathways in photosynthetic organisms, mechanisms of the regulation of these pathways, and bioconversion of photosynthetic products to hydrogen and methane. The goal of the research is to provide basic biochemical information which will make possible a better use of green plants in the utilization of solar energy to convert inorganic materials to useful organic substances. Such information is needed to guide genetic improvements of plants and microorganisms for greater productivity of biomass and improved quality of products: renewable chemicals, fuels, food, and fiber.

Specific investigations include: elucidating metabolic pathways in green cells and locating regulatory sites, the development of methods for isolating green cells from higher plant leaves to use as test systems, plant cell tissue culture and plant regeneration as a means for genetic manipulation of desired biochemical properties, maintenance of cultured cells in a differentiated state (e.g., for photosynthesis or specific biosynthesis of useful compounds like hydrocarbon), and the manipulation of microorganisms for more efficient conversion of materials from green plants.

Plant cell tissue work has been started, and is now incorporated in this task.

Donner Laboratory, LAWRENCE BERKELEY LABORATORY

49. RESONANCE STUDIES IN PHOTOSYNTHESIS 0.5
 Alan Bearden, Donner Laboratory

Application of time-dependent electron paramagnetic resonance (EPR) spectroscopy to determination of structure-function relations of photochemical steps in green-plant photosynthesis. By using newly-developed methods of EPR data acquisition, with special attention to making measurements of spin relaxation for paramagnetic oxidation-reduction components, we plan to sort out the role of various electron donors and acceptors in these photochemical reaction centers. Our methods make use of saturation-recovery and direct detection resonance techniques and the use of oriented membrane samples prepared by drying under controlled atmospheres. So far our studies have shown the magnetic orientations of several iron-sulfur acceptor components and have, due to the enhanced signal-to-noise ratio, shown several new paramagnetic components whose specific roles are being further investigated. Careful application of these new techniques has also shown that many of the before-assumed components may lie in new positions in the electron-transport chain on the acceptor side of Photosystem I. In addition, these techniques show promise in studies of the oxygen-evolving complex of Photosystem II in vivo. The extension of these studies to iron-sulfur components in the electron-transport chain of oxidative phosphorylation is also planned. This research has as its goal the elucidation of reaction pathways for the photochemical steps in photosynthesis and associated bioenergetic processes.

MICHIGAN STATE UNIVERSITY - MSU/DOE Plant Research Laboratory
East Lansing, Michigan 48824 Total Funding: \$1,250,000

50. REGULATION OF FLOWERING 2.2
 Anton Lang

Whereas evidence for hormone-like (translocatable) flower promoters ("florigen", "floral stimulus") has been available for over 40 years, unequivocal evidence for analogous, potent inhibitors of flowering ("anti-florigen") has been obtained only recently, by Task 001. This discovery markedly affects our ideas about the regulation of flowering. Failure of plants to flower under certain environmental conditions has been attributed to the lack of promoters of flowering; now we see that it may be caused by the presence of inhibitors of flowering. The inhibitors have been shown to share certain physiological properties with the promoters: they are not specific in a taxonomic nor a physiological sense, apparently being the same in different species and genera, and in different environmental (photo-periodic) response types. Further work on Task 001 is directed at the question of how wide-spread anti-florigen is in seed plants, and at its isolation and identification.

Flower formation is of great fundamental as well as at least potential practical interest--fundamental because it involves a profound alteration of the growth pattern of the plant, namely, cessation of vegetative growth and "metamorphosis" of leaves and stems into seemingly new organs, the flowers; practical because flowering is the first stage of reproductive development in seed plants and thus the premise for fruit and seed production. It can be considered as a problem of energy partitioning in the plant. For these reasons, research on regulation of flowering, including flowering inhibitors as a new "element" in this regulation, is highly pertinent to the PRL research program.

51. THE SACCHARIFICATION OF WOOD AND WASTE CELLULOSE 3.5
 Derek T. A. Lamport

A simple economic method of "cracking" cellulose to yield feedstock sugars remains elusive.

This task explores a novel method for "cracking" cellulose using gaseous or liquid anhydrous hydrogen fluoride at circumambient temperatures and pressures. The method leads to cellulose saccharification and has the advantage over other proposed processes of speed and simplicity. Furthermore theory predicts that the HF can largely be recycled. Therefore we shall determine saccharide yields, and fluoride retention, as a function of time, temperature and evaporative technique.

With optimized reaction conditions we can proceed to process design and possible scale-up to pilot plant level.

MICHIGAN STATE UNIVERSITY - MSU/DOE Plant Research Laboratory - continued

52. ENVIRONMENTAL CONTROL OF PLANT DEVELOPMENT AND
ITS RELATION TO HORMONES
Jan A. D. Zeevaart

4.0

Environmental factors such as daylength, temperature and water deficits have pronounced effects on plant growth and development. The objective of this task is to study the role that hormones play as intermediaries between the perception of an environmental factor and the morphological manifestation in the following cases:

(a) Flower formation which is induced by the flower hormone. The major question remains the nature of this hormone. Two approaches are being pursued: phloem exudate is being tested for biological activity, and the chemical composition of exudate from flowering and vegetative plants is analyzed and compared.

(b) Stem and leaf growth in rosette plants as regulated by gibberellins (GAs). The goal is to determine what kind of changes the photoperiod causes in the GA status that ultimately result in stem elongation.

(c) Wilting of leaves which is due to a water deficit and is associated with accumulation of abscisic acid (ABA) and its metabolites. Conversely, relief of stress results in rapid degradation of excess ABA. The aim is to determine how ABA synthesis is enhanced in stressed leaves, as well as to learn the sites of ABA synthesis and metabolism.

Projects (a) and (b) are concerned with optimizing the conversion of solar energy into biomass, while (c) deals with adaptation of plants to environmental stress.

MICHIGAN STATE UNIVERSITY - MSU/DOE Plant Research Laboratory - continued

53. PHOTOSYNTHETIC PARTITIONING OF ASSIMILATES 0.8
Jan A. D. Zeevaart
Hans Kende

This task is concerned with partitioning of assimilates. The purpose is to determine in the bean plant (i) the role of root cytokinins in the regulation of plant senescence; (ii) the role of hormones in the flow of assimilates from source to sink; (iii) the pattern of assimilate distribution of different stages of development in plants grown under two different nitrogen regimes, namely supplied with nitrate, or dependent on biological nitrogen fixation; (iv) the energy requirements for nitrogen metabolism, in particular the energy needed for biological nitrogen fixation. Thus, this task is related to the over-all program of the PRL in that it is concerned with the conversion of solar energy into biomass; ultimately the results of this research may also help to design strategies that will make it possible to reduce the energy input into crop production in the form of chemical fertilizer.

54. DEVELOPMENTAL BIOLOGY OF NITROGEN-FIXING ALGAE 6.9
Coleman P. Wolk

This task is concerned with the fixation of atmospheric nitrogen (N_2) by filamentous cyanobacteria (blue-green algae), and with the closely related phenomena of cellular interactions and differentiation in these organisms. Special cells called heterocysts fix N_2 , in cooperation with nearby vegetative cells, inhibit vegetative cells from becoming heterocysts, and induce them to become spores. The following projects are currently pursued: (1) Mutants affected in nitrogen fixation, development, photosynthesis, and intermediary metabolism having been isolated, genetic transfer is being attempted. (2) We are elucidating the principal distinguishing biochemical characteristics of heterocysts and spores, including the pathway of electron transfer to nitrogenase and the structure and biosynthesis of envelope constituents. (3) We are trying to identify the interactions by which vegetative cells supply reductant for N_2 fixation in heterocysts, and by which heterocysts induce adjacent cells to sporulate. This entire research is related to the problem that most crop plants depend upon supply of nitrogen fertilizers, the production of which requires substantial energy expenditures. The long-range goal is to provide nitrogen for plants by photosynthesis, possibly through symbiosis with N_2 -fixing cyanobacteria, rather than by use of natural gas.

MICHIGAN STATE UNIVERSITY - MSU/DOE Plant Research Laboratory - continued

55. PLANT GROWTH REGULATIONS BY HORMONES (CYTOKININS, 3.7
GIBBERELLINS, ETHYLENE)
Hans Kende

The principal objectives of this task are to gain knowledge on the mode of action of the plant hormones cytokinin and ethylene. These hormones regulate processes related to senescence and stress in plants, ethylene promoting aging and symptoms of stress and cytokinins retarding them.

a) Regulation of aging in plants by ethylene and cytokinins: Of particular interest are degradative processes which affect cell membrane integrity and cellular compartmentation. Membrane breakdown leading to irreversible deterioration of the cell is promoted by ethylene and retarded by cytokinins. Investigations will also focus on the mechanism by which ethylene synthesis is regulated in plants, either by ethylene (positive feedback) or by stress.

b) The site of action of cytokinins will be studied by investigating the binding of radioactive hormone or a radioactive cytokinin photoaffinity label. In order to assess the significance of binding sites, hormone analogs of varied biological activities will be synthesized and their binding properties measured.

The work described above is part of the Laboratory's effort to understand how stress affects plants and how plants cope with it. Work on plant senescence is also important in the context of storage and transportation of perishable agricultural commodities. Manipulation of the aging process may lead to decreased energy input in the prevention of produce spoilage.

56. REGULATION OF PROTEIN FORMATION IN PLANTS; 4.6
SIGNIFICANCE IN GROWTH REGULATION
Philip Filner

Changes in the availability of nutrient sources are among the most frequently encountered environmental challenges with which plants must cope. The three major nutrients which are reduced, with investments of large amounts of energy, are carbon, nitrogen and sulfur. The paths of nitrogen and sulfur assimilation are functionally convergent, in that both elements are used largely to make proteins. Cultured plant cells afford an excellent opportunity to study how plants cope with fluctuations in the availability of these nutrients, because the cells can be grown in chemically defined media under precisely controlled conditions, nutrient consumption and metabolism can be followed conveniently and nutrient supplies can be perturbed precisely, at will. We are analyzing the responses of cultured plant cells to perturbations of their carbon, nitrogen or sulfur supplies. Emphasis is placed on detection of regulatory phenomena involving enzyme formation. Current efforts are concentrated on responses of plant cells and plants to suboptimal nitrate concentrations, on regulatory coupling between the nitrate reduction and sulfate reduction pathways, and on slow adaptive changes in urease during prolonged growth of cells on urea.

MICHIGAN STATE UNIVERSITY - MSU/DOE Plant Research Laboratory - continued

57. ENZYMATIC MECHANISMS AND REGULATION OF PLANT

4.7

CELL WALL BIOSYNTHESIS

Deborah P. Delmer

One of the goals of the Plant Research Laboratory is to conduct research on processes that determine the conversion of solar into chemical energy (biomass) in plants. Task 004 specifically addresses this goal as it seeks, as its major objective, to determine the pathway of conversion in higher plants of reduced carbon into cellulose, the world's most abundant organic compound. For these studies, we use the developing cotton fiber as an experimental system because this cell produces a nearly pure cellulosic cell wall. Using a combination of enzyme studies, in vivo pulse-labeling procedures and specific inhibitors, we trace the pathway of conversion of ^{14}C -glucose into ^{14}C -cellulose.

Other related projects included in this task are: 1) a biochemical analysis of the process of cell wall regeneration in protoplasts, a process which represents one of the critical early steps in the genetic engineering of plants involving protoplast fusion techniques; 2) identification and structural characterization of intracellular high molecular weight precursors of non-cellulosic cell wall polysaccharides in cultured plant cells; 3) use of a solute-exclusion technique to allow a determination of the pore size of plant cell walls, a size which sets limits upon the effective molecular size of toxins, elicitors, herbicides and nutrients available to plants, and 4) a study of the process of synthesis of legume storage proteins--a major source of protein in man.

58. RESISTANCE OF PLANTS TO ENVIRONMENTAL STRESS

4.1

Andrew Hanson

This task investigates the metabolic basis for injury from, and adaptation to, environmental stresses--specifically water stress--with the long-range objective of developing novel selection methods for stress-adaptation applicable in plant breeding. Accordingly, research is conducted in collaboration with the plant-breeding program of Dr. E. H. Everson (Crop and Soil Science Department, MSU), and has the following aims: (1) to characterize the metabolic responses of plants elicited by water stress; (2) to assess genetic variation for these responses; (3) to evaluate the adaptive value of responses by physiological and genetic experiments; (4) to estimate the bioenergetic costs of the responses. One metabolic response to water stress in several cereals and other crops is accumulation of glycine betaine; this and other metabolic consequences of water deficit are being examined from the standpoint of possible adaptive value, and as potential indices of drought-injury or -resistance in plant breeding. Test objects are whole plants and mini-crops of barley, other cereals and their wild relatives, and grain legumes.

MICHIGAN STATE UNIVERSITY - MSU/DOE Plant Research Laboratory - continued

59. ACQUISITION OF ENVIRONMENTAL INFORMATION
THROUGH LIGHT PERCEPTION

4.4

Kenneth L. Poff

This task seeks to understand mechanisms of acquisition of environmental information, in several types of organisms as a basis for understanding sensory transduction in plants. Non-visual light and temperature perception are under investigation. We are studying light sensing in the cellular slime mold Dictyostelium in which we have identified several photoreceptor pigments, and for which we have been able to formulate an approach using mutant selection and analyses. We are also studying the "blue light" photoreceptor system(s) which control(s) numerous light responses in plants, by using specific inhibitors as probes into the initial steps in the transduction sequence. Temperature perception is being studied in two systems: thermotaxis by pseudoplasmodia of Dictyostelium and the sensing of low temperature by cultured cereal cells prior to the acquisition of frost hardiness. Thermotaxis is a unique model system for the study of biothermometry because of the extreme sensitivity to temperature of the pseudoplasmodia, the narrow temperature-range over which temperature is measured, and the phenomenon of adaptability--the dependence of the thermotaxis temperature range on the previous growth temperature of the cells.

Non-visual light perception is a plant process which determines the conversion of solar energy into chemical energy through various mechanisms including control of the array of leaves to light. Temperature perception is one aspect of the resistance of plants to environmental stresses.

MICHIGAN STATE UNIVERSITY - MSU/DOE Plant Research Laboratory - continued

60. ANALYSIS OF PHOTOSYNTHETIC MEMBRANES
Charles J. Arntzen

1.5

The objective of this task is to understand structure and function of internal lamellae (thylakoids) of higher plant chloroplasts, which are of primary influence in regulating photosynthetic quantum efficiency. Questions asked are:

- a) What (and how many) proteins comprise the reaction-center complexes of photosystems I and II? Detergent-isolated complexes reconstituted into lipid vesicles are used to test for function in parallel with protein characterization. The goal is to understand interactions among proteins and how these regulate electron transport efficiency.
- b) Do specific lipid-protein interactions influence reaction-center and pigment-protein stability? This will be tested by analyzing for specific "boundary lipids" in isolated complexes, and by fusing "foreign" lipid vesicles with chloroplasts to change specific protein/lipid ratios. The goal is to understand processes that maintain long-term efficiency of photosynthetic light reactions.
- c) Are there specific regulatory controls for chloroplast membrane biosynthesis (assembly), and turnover, and for short-term adaptation processes? The approach will be to analyze rapidly turned-over thylakoid proteins in mature leaf tissue, determine their site of localization, and characterize their function where possible. We will also analyze post-translational steps of protein modification. The goal will be to identify possible control steps by which photosynthetic performance can be extended or maximized in intact plants.

UNIVERSITY OF PUERTO RICO
Center for Energy and Environment Research
San Juan, Puerto Rico

Total Funding: \$35,000

61. DESULFURIZATION OF ORGANO-SULFUR COMPOUNDS AND PETROLEUM FRACTIONS BY MICROORGANISMS 1.0
Juan J. Rigau

It is proposed to continue with the isolation and characterization of indigenous microorganisms and assessment of anaerobic-aerobic dual systems involved in the degradation of organic sulfur compounds in petroleum and non-conventional heavy oil fractions. The technical approach involves:

- (1) Selection of microorganisms.
- (2) Determination of the optimal growth conditions for mass culture.
- (3) Definition of optimal reaction conditions.
- (4) Perform laboratory scale and field demonstrations.

In particular the proposed research will include (a) more comprehensive group type, analysis of the major components of oil before and after biotreatment; (b) detailed consideration of a number of parameters such as temperature, pH, salt effects, and nutrient limitations including the interaction of all of the above factors; and (c) laboratory scale modelling studies required to help establish some of the physical and chemical constraints associated with the inoculation of microorganisms in an oil reservoir.

Our primary intention is to study the extent and full effects of bacteria in a petroleum reservoir establishing the basic criteria for evaluation of amenability of oil formations to bacteria treatment. In this regard detailed mechanisms will be developed for joint efforts with the Venezuelan Institute for Petroleum Technology and the Ministry of Energy and Mines.

SOLAR ENERGY RESEARCH INSTITUTE
1536 Cole Boulevard
Golden, Colorado

Total Funding: \$117,000

62. PHOTOCOVERSION

2.0

Michael Seibert, Research Division
Paul F. Weaver " "
Stephen Lien " "

This proposal outlines a three Task research program in the general area of photobiological H₂ production. Task 1 will use biochemical and genetic techniques to identify the electron transport components and pathways associated with hydrogenase activity in photosynthetic bacteria. The ultimate goal is to determine the feasibility of obtaining an organism which evolves H₂ via a hydrogenase rather than the normal nitrogenase pathway. Task 2 will focus on the purification and characterization of algal hydrogenase. This information is necessary to isolate or construct genetically an organism which will evolve H₂ under oxygenic photosynthetic conditions. Task 3 will seek to understand the properties of bacterial reaction center complexes in monolayer and multilayer stacks. These assemblies could serve as a model for or even "building blocks" for an in vitro H₂ evolving system.

The microbial energy conversion program will be expanded to encompass the biochemistry and physiology of hydrocarbon (HC) production in algae. The objectives of this work will be to define the metabolic pathways involved in HC production and to determine the control mechanisms that lead to HC production under certain naturally occurring conditions.

UNIVERSITY OF TENNESSEE
Comparative Animal Research Laboratory
1299 Bethel Valley Road
Oak Ridge, Tennessee 37830

Total Funding: \$356,000

63. PLANT BIOMASS AND ENERGY CONVERSION

6.0

M. J. Constantin
D. E. Foard
F. J. Ryan

Recently fixed carbon of plant biomass (stored solar energy) is a unique renewable resource as a nonconventional energy source. The biological limits for the exploitation of biomass under existing agricultural and economic constraints must be determined and ultimately expanded. Furthermore, the mechanisms and magnitude of pollutant effects on biomass production must be determined.

Research objectives are to (1) better understand biological processes related to plant growth and development (biomass production), (2) develop screens to identify mutants with characteristics that can be used to improve biomass yield and quality, and (3) develop higher plant genetic systems for monitoring environmental mutagens.

We are studying the mechanism of energy-related pollutants' effects on photosynthesis; feasibility of genetically manipulating photosynthate partitioning; regulation of synthesis and accumulation of essential amino acids in cereal grains; feasibility of screens to identify mutants to improve biomass productivity and quality; transport of basic amino acids in cultured tobacco cells; mRNA (isolation, characterization and quantitation) for low-molecular-weight protease inhibitors and cDNA (preparation) to isolate specific gene segments, RNA transcription products, and to determine nucleotide sequences; and use of higher plant genetic systems to monitor the environment for mutagens.

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