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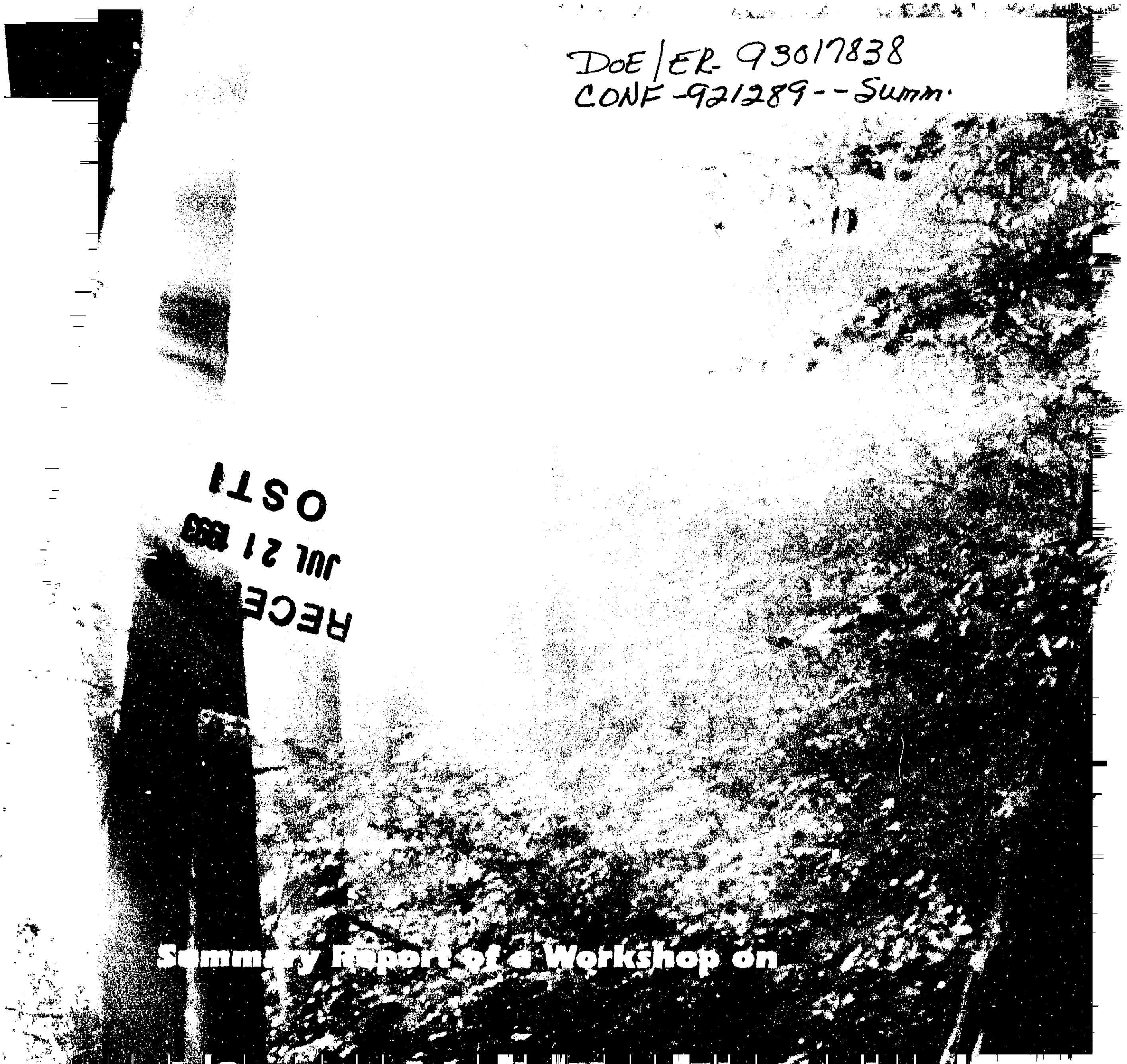

Summary Report of a Workshop on
search Opportunities in Plant Biochemistry

NOTE

The workshop on the needs and opportunities in plant biochemistry was organized by Dr. Chris Somerville of the Michigan State University/Department of Energy/Plant Research Laboratory. The convergence was sponsored by the Division of Energy Biosciences of the Office of Basic Energy Sciences of the Department of Energy.

One objective of the workshop was to bring forth ideas about how plant biochemistry research and training might benefit the peoples of the world. The ideas outlined are aimed definitely at general efforts with no limitations to specific programs, agencies or countries. If anything, the objective is more collaboration and cooperation with less competitiveness in building a broader foundation of plant biochemistry knowledge.

The writing and editing of this workshop report was performed predominantly by Dr. Somerville, with some assistance from Dr. Robert Rabson of the Division of Energy Biosciences.

It is the hope of all those who were involved in the workshop, and many others, that the report will alert people to the importance of the broad topics and to arouse sufficient interest on the part of current and potential researchers so that more of the questions will be addressed. It should also be recognized that while the scope of the workshop was broad, it was not inclusive of every topic area in plant biochemistry.

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Summary Report of a Workshop on

Research Opportunities in Plant Biochemistry

December 11-13, 1992
Kona, Hawaii

DIVISION OF ENERGY BIOSCIENCES
OFFICE OF BASIC ENERGY SCIENCES
OFFICE OF ENERGY RESEARCH
U.S. DEPARTMENT OF ENERGY

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TABLE OF CONTENTS

PART I

PART II

EXECUTIVE SUMMARY

A DOE-sponsored workshop was held December 11-13, 1992 in Kona, Hawaii to discuss those aspects of fundamental research in plant biochemistry deemed essential to provide the basic information base necessary for exploiting plant biotechnology to meet future societal needs. Twenty nine scientists, with interests representing many of the various areas of plant biochemistry, participated (Appendix I). The workshop was intended to define in both broad and specific terms the current state of knowledge in the general area of metabolic biochemistry, and to identify those areas that afford unusual opportunity or that are relatively underdeveloped in comparison with other areas of plant biology. Participants provided critiques of the state of knowledge of the major areas of metabolic biochemistry in relation to a series of questions that are presented in Appendix II.

The substance of the discussions concerning these and related topics are summarized in the following report. Because of the breadth of the topic and, in some cases, the diversity of opinion, no attempt was made to arrive at a consensus. Each of the eight sessions of the workshop was recorded by two rapporteurs selected from among the participants. The following report is largely derived from their notes and from comments made during the workshop. Other highly knowledgeable non-attendees also contributed to this report in the form of comments on an earlier draft.

One of the general conclusions from the discussion was that certain areas of plant biochemistry are developing at a satisfactory pace whereas other areas of strategic importance and apparent opportunities would benefit from greater attention. The areas of greatest opportunity, in this respect, included the following:

- Metabolic engineering based on the use of transgenic plants to test theories concerning the mechanisms that regulate biochemical pathways and carbon and nitrogen partitioning processes.
- Elucidation of the mechanisms involved in biosynthesis and extension of primary and secondary cell walls, and the relationships between structure and function of cell wall components.
- Elucidation of the mechanisms that regulate the structure of various starches.
- Characterization of the enzymes and corresponding genes that catalyze the biosynthesis and modification of fatty acids, and identification of the mechanisms that regulate lipid biosynthesis.
- Elucidation of the mechanisms which regulate the translocation and accumulation of carbon and nitrogen between different cellular compartments and tissues.
- Characterization of the enzymes and genes for all of the steps in at least one representative pathway of terpenoid biosynthesis, and the use of these materials to determine the mechanisms that

regulate flux through the pathway.

- Elucidation of the mechanisms that regulate the tissue-specific and stress-induced expression of biochemical pathways with particular reference to pathways leading to secondary metabolites.
- Elucidation of biochemical mechanisms involved in perception, response and adaptation to the environment. This involves the identification of signal molecules, characterization of the components of signal transduction pathways and the description of metabolic and structural changes in response to biotic and abiotic stresses.

Each of these areas poses unique conceptual and technical problems. However, it was concluded that because of technical innovations, advances in genetic technology and relevant advances in the biochemistry of animals and microorganisms, many previously intractable problems have become accessible. In particular, the development of new analytical instrumentation and methods for solubilizing membrane proteins has greatly facilitated the purification of proteins. The development of facile methods for producing transgenic plants of many species has opened up the possibility to directly test the role of key enzymes by altering flux through pathways by over and underexpression of these enzymes. Similarly, the proliferation of methods for identifying transcriptional regulators was seen as creating novel opportunities for the manipulation of secondary metabolites providing that the isolation of genes for biosynthetic enzymes develops apace. The explosion of protein sequence information and the advent of large-scale cDNA sequencing provides new opportunities to study key enzymes by working backwards from the gene to the protein. Similarly, the development of several excellent genetic model systems such as *Arabidopsis*, tomato and maize provides new approaches to isolate genes for many enzymes. As one participant noted:

"*Because of its small size Arabidopsis* is not an attractive organism for biochemists accustomed to large quantities of material. However, because of the genetic tools available in *Arabidopsis*, it would be unwise not to use it."

However, as another participant noted:

"Because of the important differences which may occur between species it is not appropriate to focus on one model species."

This comment generated a lively discussion concerning the need for balance between understanding one model system in depth and the need to understand the range of possibilities. There was agreement that the use of model systems facilitates rapid progress on certain problems. However, since model systems necessarily represent only a subset of biological phenomena, it is essential to study a range of species.

The importance of exploiting model systems was apparent in considering the overwhelming diversity of the chemical constituents of plants. It is not feasible to characterize the biosynthetic pathway and the associated enzymology of all secondary metabolites.

In this respect, it was suggested that for major classes of compounds such as terpenoids, it may be useful to identify a suitable model species, such as tomato, that would permit a molecular genetic analysis of at least one representative biosynthetic pathway. Because genes for enzymes that catalyze similar reactions may have adequate homology so that they cross hybridize, an effort should be made to isolate genes for representatives of the major classes of biosynthetic reactions. This would facilitate access to genes and enzymes from divergent biochemical pathways. Another initiative that would enhance access to the chemical diversity of plants is the development of computer databases which would provide access to the available information on the chemical constituents of plants.

In considering the career opportunities for young scientists, many participants expressed concern about the limited numbers of permanent research positions available in plant biology. However, there was unanimity concerning the need to recruit excellent students into plant biochemistry to ensure the future vitality of the discipline. It was generally agreed that the emphasis should be on increasing the quality of graduate students in plant biochemistry rather than encouraging large increases in number. The most effective mechanism for increasing the proportion of high quality students would be to institute a graduate fellowship program in plant biochemistry, as well as providing postdoctoral fellowships designed to provide opportunities for chemists, biochemists and others trained in animal and microbial biochemistry to gain experience in plant biochemistry.

It is hoped that the report will stimulate discussion within the plant biology community and others with interest in research and will serve to highlight some of the exciting scientific opportunities in relatively undeveloped areas.

INTRODUCTION

Why study plant biochemistry? The fact that human life and virtually all other forms of life are totally dependent on plants should be a sufficient incentive. Plants, including algae, are not only a food source for humans but also provide feed for domestic and wild animals as well as for aquatic life. Plants also serve as the fuel resource for most cultures. This includes the developed world if one considers that plants were the origin of most of the fossil fuels upon which we now depend. Plants provide fibers for clothing, paper, rope and timber, and are the sources of many medicines and pesticides. Many other industrial products such as detergents, plastics, rubber, lubricants, waxes, paints and coatings, dyes, tannins, gums, resins, solvents and industrial feedstocks are also derived from plants. Also, plants play a crucial role in the stabilization of the environment by preventing excess erosion, by cleansing the air and by absorbing certain pollutants in soils and aquatic locations. It is somewhat paradoxical that despite the broad and vital role that plants play in human life, the chemistry and biochemistry of plants has received comparatively limited attention. As several participants noted:

"The importance of plants has not been well stated. Both scientists and non-scientists have not been persuaded of the many reasons for acquiring a thorough understanding of plant biology."

Clearly, the more than 300,000 species of higher plants represent a resource that until now has generally been utilized in a strictly empirical manner. Recognition is now growing of the fact that plants collectively have the capacity to synthesize a colossal spectrum of potentially useful compounds and that it may be possible to greatly expand the ways in which higher plants are utilized. One participant summarized the opportunities:

"Plant research has been linked too closely to conventional agricultural production of food. However, because of new technologies it is now possible to create entirely new agricultural commodities that can be used as renewable sources of biomaterials that will reduce our dependence on petroleum and simultaneously reduce or eliminate the social and economic costs associated with current overproduction of a limited number of commodities."

The impetus for considering more efficient and expanded uses of higher plants arises from the development, during the past decade, of methods for their rational genetic modification. This technology has matured to the point where it is now possible to control rather precisely the timing, tissue-specificity and level of expression of introduced genes. Thus, genetic technology is no longer the limitation in considering possible applications. Rather, in most cases, it is the large gaps in our knowledge of plant biochemistry and related disciplines that pose the greatest limitation. These gaps in our knowledge of plant biochemistry have several root causes which have been noted previously by others (see the general preface to "The Biochemistry of Plants" by P.K. Stumpf and E.E. Conn, Academic Press, 1980). One of the most problematic was summarized as follows by one commentator:

"Far too many people are allowed to believe that, photosynthesis apart, plant biochemistry is the same as that in other eukaryotic cells. The unique compartmentation of plant cells sets them apart and we cannot use knowledge of animal biochemistry to determine what happens in plant cells."

Thus, while an integration of relevant knowledge gained from animal and microbial systems is critical, the basic knowledge of plants, particularly their biochemistry, must be developed as the foundation for new biotechnological developments.

The purpose of the current workshop was to outline many of the existing limitations in plant biochemistry that must be addressed to promote progress towards future developments. The workshop was organized by bringing knowledgeable representatives of numerous research areas together to discuss what is needed in plant biochemical research generally and also with specific reference to the different categories of chemical constituents found in plants. The discussions took the form of introductory descriptions of fields followed by open discussions of many different points that were raised. Some of the participants were asked to serve as rapporteurs of the different sessions. The remarks offered by the rapporteurs have been edited and are presented in this report along with some quotations. In addition, an early draft of this report was circulated to the workshop participants along with a substantial number of other highly knowledgeable individuals asking for their comments. The ideas and suggestions received in response have been incorporated, where possible, into this final revised version.

In addition to the specific topic areas, there was discussion of general issues such as the need to encourage involvement of young scientists in plant biochemistry. Some of the general items that were raised are listed below. Each of the participants received a letter prior to the meeting that contained a brief discussion of the aims of the workshop in order to provide a chance to contemplate the issues before the meeting. A copy of theses topics is presented as Appendix II. A listing of the participants is provided in Appendix I.

THE RELATIONSHIP BETWEEN BIOCHEMISTRY AND BIOTECHNOLOGY

The conceptual and technical framework that defines plant biotechnology is a significant departure from, but related to, the traditional agricultural disciplines of plant breeding, horticulture and agronomy. (Note: The term "biotechnology" as used in this context implies the application of molecular biological procedures aimed at defining mechanisms.) A crucial difference is that traditional methods of plant improvement did not depend upon detailed mechanistic knowledge of the underlying biology of a plant trait. By contrast, the techniques of biotechnology require a detailed understanding of the mechanistic basis for plant traits. Since many applications of plant biotechnology involve the genetic manipulation of biochemical pathways, it is critical to acquire detailed information about many pathways that have hitherto received relatively cursory attention, or none at all. Furthermore, although it is common to speak of different biochemical pathways, the cell functions as an integrated unit in which the various pathways interact through complex regulatory mechanisms that operate at various levels from allostery to gene transcription. For this reason, a frequent problem hindering the application of plant biotechnology is a lack of specific information about the ancillary metabolic consequences of a genetic modification. It is a common experience, of those working in the field, to engage on a seemingly straightforward modification of a plant by the addition of one gene only to discover that progress becomes limited by a complete lack of knowledge about an interacting pathway. This common difficulty imposes a risk in the commercial applications of biotechnology because of the difficulties in assessing the costs associated with solving problems adjacent to the specific objective.

Another critical difference between biotechnology and plant breeding is that traditional plant breeding concerns the utilization of genetic variation within a species or close relatives, and more recently, the possible induction of a desired mutant. By contrast, because of the ability to move traits from one organism to another, plant biotechnology exploits a much broader range of organisms. Thus, many characters which are only found in wild species have assumed new importance. It is now possible to envision that these traits may be transferred from wild species that are not suited for cultivation, into agricultural, horticultural or forestry species. This provides a new and tangible reason to maintain and understand plant diversity. Only a fraction of the estimated 100,000 compounds which occur in higher plants have been characterized, including 22,000 terpenoids, a few thousand carbohydrates and a broad spectrum of other substances. This tremendous chemical diversity reflects the existence, in different plant species, of tens of thousands of functionally different enzymes. As the concepts and goals of plant biotechnology evolve, this storehouse of enzymatic catalysts can provide the raw materials for many new applications in both plant and non-plant biotechnology. In many instances plant genes will be used to produce novel compounds by expression in microbial hosts and vice versa.

The issues pertaining to mechanisms that promote the maintenance of plant diversity are beyond the scope of this report. However, there was unanimity that efforts to conserve biodiversity are an essential aspect of support for the discipline of plant biochemistry and biotechnology. In this respect, the training of taxonomists and phytochemists skilled in the characterization of plant constituents represents a tangible action that may help ensure the recognition and conservation of plants with unique chemical constituents. It was also noted that extensive germplasm collections for crop species represent valuable resources for plant biochemistry. In a number of instances it has been found that different accessions of the same species contain significantly different amounts of certain metabolites or structural compounds. These differences have been very useful in examining the functional significance of particular plant constituents and may facilitate the characterization of the relevant enzymes and genes.

As a final point it should be noted that, although plant biotechnology is inevitably directed towards commercialization of plant-derived products, it is inappropriate to expect industry to provide the bulk of support for basic research in the underlying scientific disciplines, or to be held responsible for basic research that may lead directly to applications. Thus, in the review of grant proposals, careful attention must be paid to avoid the negative connotations that have traditionally been associated with research which is associated with applied goals. As one participant noted:

> ***"Having an applied goal does not necessarily harm basic research - much the contrary. A focus on an applied goal frequently forces one to address difficult problems that would be avoided if one was merely following the path of least resistance as is frequently the case in curiosity driven research."***

The reason for this is that the horizon for industry is typically short relative to the time required to solve many fundamental biochemical problems. Thus, reliance on industry to establish the research agenda in basic sciences will inevitably result in serious gaps in knowledge because of unexpected developments or changes in priorities. Plant biotechnology is a powerful tool which has great possibilities for societal change. Therefore, mechanisms must be in place to ensure public support for innovative basic research in the academic disciplines that underpin biotechnology, without having such support tied to arguments for short term utility.

A good example of this is the major fundamental genetic discoveries made with plants over many years that subsequently laid the foundation for understanding genetics and cytogenetics, including human genetics as it relates to health.

AN IMPORTANT ROLE FOR COMPARATIVE AND ECOLOGICAL BIOCHEMISTRY

Most biologists study bacteria, fungi and animals. Consequently, knowledge of the fundamental processes in these organisms has advanced much more quickly than in plants. With a few possible exceptions, all of the ubiquitous biochemical pathways have been originally described in microbes or animals. Because of the lower value traditionally attached to comparative biochemistry, and the relatively low level of support for plant biochemistry compared to animal biochemistry, there has been a disincentive for biochemists to investigate the ubiquitous pathways in plants. The fact that the plant pathways are frequently similar to those in animals or bacteria appears to have discouraged detailed characterization of the pathways in plants. By contrast, some aspects of biochemistry that were relatively unique to plants, such as photosynthetic carbon dioxide fixation, have been characterized at a similar level of refinement to that of the central pathways in animals. As noted above, there are now tangible reasons why it is now desirable to acquire a detailed understanding of many pathways in plants. Furthermore, as was repeatedly stressed during the meeting by various participants, in order to know how broadly useful detailed knowledge about a particular pathway is, it is also essential to acquire comparative information about the differences between plant species. As one participant noted:

"It is frequently the differences between species that serves to highlight the existence of an interesting biological phenomenon."

For example, various members of the same plant family may synthesize very different molecules as biochemical defenses against the same biotic or abiotic stresses. Such diversity among quite closely related plants can provide important clues about which pathways are most amenable to metabolic engineering for pest and stress resistance.

In many cases the most efficient mechanism for increasing the base of comparative information would be to encourage and facilitate cooperation among researchers working on similar problems in different species and to foster exchange of information and biological materials. Recently, there has been an effort to enhance such interchanges through the establishment of networks (a segment of the DOE/NSF/USDA Joint Program on Collaborative Research in Plant Biology). In addition to increasing communication among dispersed research groups, networks can be used to expand the range of publicly available metabolic intermediates, enzymes, antibodies and reference compounds that will stimulate research in underdeveloped aspects of plant biochemistry. Special consideration should be accorded research grant proposals that propose to perform a specialized service function in this respect.

Because the literature concerning the chemical constituents of plants is vast and dispersed, it has become essential to develop computer databases that will provide access to the occurrence and distribution of the chemical constituents of plants. As an example, databases such as Genbank have become absolutely essential in studies on gene structure. More recently, another database, CarbBank, is proving to be a powerful aid in carbohydrate studies. Other databases such as the PHYTOCHEMCO database of the USDA National Germplasm Laboratory appear to be less widely utilized, possibly due to the lack of an effective mechanism for informing the broader scientific community about the availability of these resources. The Federal agencies should convene a workshop to discuss this complex issue. The availability of comprehensive databases would stimulate activity in comparative biochemistry by reducing the amount of effort required to identify scientific questions and to locate the necessary plant material. Such databases could also be powerful educational devices for developing an appreciation of plant diversity in students and their teachers. These databases also enhance more collaboration among scientists in different countries both in consolidating and distributing the information.

CAREER OPPORTUNITIES IN PLANT BIOCHEMISTRY

Academic research in science is performed principally by graduate students and postdoctoral associates. It is mostly grant support that is used to pay the stipends of these individuals. When the question arises as to how to enhance interest and productivity in an important field, the inevitable answer is to increase funding so that more students and postdoctorals can be attracted to approach significant unsolved problems. Ultimately, however, students and postdoctorals aspire to career positions. Therefore, in considering whether support for more people should be recommended, one must consider whether there are positions to accommodate the resulting increase in flux of students and postdoctorals. Career opportunities for biologists are not narrowly defined. It is not uncommon for a student trained in one biological discipline to obtain a career position in another discipline. However, this flexibility varies from one area to another depending upon the perceived general utility of a particular type of training. In this regard, career prospects for well trained plant biochemists appear to be quite bright at present and for the foreseeable future based on the key role in furthering biotechnology development. However, it must be added that the best prospects are for individuals who have been trained in both biochemistry and molecular biology.

At the same time, it must be recognized that plant biochemistry is a critical basic topic that extends well beyond its relevance to immediate biotechnology. During recent times, interest in plant biochemistry has lagged significantly and because of retirements during this decade the pool of biochemists appears to have contracted significantly. Part of the reason for the decline in interest in plant biochemistry is probably related to the general trend in biology as a whole.

One participant commented that there is also a tendency among universities to pursue replacements among faculty in the most fashionable disciplines as molecular biology rather than areas such as plant biochemistry. This could result in a certain amount of neglect of plant biochemistry with respect to both education and research if new faculty lack a reasonable background.

Great progress in defining metabolic pathways was made during the "golden age" of biochemistry in the middle part of this century. There are few, if any, ubiquitous pathways which are not at least partially defined. This has reduced the incidence of discovery in many aspects of metabolic biochemistry. Even the discovery of a completely novel reaction mechanism does not elicit broad interest in the biological community. By contrast, the problems concerning mechanisms of development, signal transduction and the regulation of gene expression are perceived as providing greater opportunities for the discovery of new principles.

Because plant biologists are necessarily attuned to developments in other biological disciplines this has a significant effect on what is considered an acceptable problem in plant biology. Students contemplating a career in plant biology perceive these issues and are attracted to developing areas where there is a tangible sense of excitement and discovery. As noted in the subsequent sections, many areas of plant biochemistry offer exciting opportunities to exploit recent developments in molecular and cell biology. However, because the pool of plant biochemists is now relatively small, and because there has not been effective dissemination of the exciting opportunities, there will be a continuing problem in recruiting the best and brightest students without steps taken to rectify the situation. The most effective way of addressing this need would be to institute a program of graduate and postgraduate fellowships similar to the very successful "NSF Postdoctoral Fellowships in Plant Biology" or fellowships of the "Life Sciences Research Foundation". The importance of this approach is that it serves to focus attention on the discipline and can place appropriate emphasis on recruitment of students and postdoctorals in chemistry and in other non-plant science disciplines. Because fellowships provide a considerable degree of independence to promising students and postdoctorals, this mechanism could also be a useful way of engendering research in underdeveloped areas. It was also suggested that fellowships to bring undergraduate students into plant biochemistry laboratories via summer internships could be an effective way, in the long run, of expanding interest in this important area.

Another aspect relating to the attraction of highly competent academic researchers to particular problem areas relates to both the fashionability of the area and the rate at which advances may be made in areas with limited background information. For a young researcher confronted with obtaining research support and for attaining academic tenure, plant biochemistry in recent years has not fit easily within the fashionability category nor has substantially demonstrable progress always come quickly. Thus, one suggestion that emerged was the encouragement of established scientists who already have tenure and some other support to seek novel scientific opportunities to pursue. To promote this, it might be useful to have a small number of awards specifically for mid-career scientists who propose to pursue a new avenue of research on an underdeveloped topic. This may be a particularly effective mechanism for generating new research initiatives on difficult problems. This by no means should imply that young investigators be excluded from involvement in underdeveloped research topics. Established investigators are generally better able to absorb the risks inherent in initiating research in underworked areas and possibly on intractable problems.

Another suggestion made during the sessions was that some efforts ought to be made to introduce younger students in high schools and other lower levels to plant biochemistry. A program similar to "Fast Plants", which is now being used in many schools, might be a means of accomplishing this generation of interest among young fellows along with introducing them to science generally.

FUNDING FOR PLANT BIOCHEMISTRY

Research in plant biochemistry is primarily supported by programs within the DOE, USDA and NSF. These agencies are considered to be supportive of excellent proposals concerning plant biochemistry. In some cases biochemistry proposals are not reviewed by individuals who possess the degree of expertise in the discipline to identify the most promising ideas. It would be useful to acknowledge formally this fact by assuring that programs include biochemists as panel members. Similarly, because the NIH review system does not rely on ad hoc reviews, it would be useful to have plant biochemists represented on the Biochemistry and Physiological Chemistry study sections. It might also be useful for one of the scientific societies, such as the American Society of Plant Physiologists, to present a formal position paper to the NIH explaining the importance and opportunities in plant biology relating to health.

Because the field of plant biochemistry is relatively small and dispersed, the few centers of excellence that do exist should be strongly encouraged to develop research training programs at the student and postdoctoral levels. As a complement to this is the recent DOE/NSF/USDA program of coordination grants to foster cooperative activities among geographically separated researchers by providing funds to sponsor workshops, the development of electronic newsgroups, the synthesis of experimental reagents and the development of specialized databases. As one participant noted:

"Such collaborative and interdisciplinary mechanisms are very important in preventing different laboratories from reinventing the wheel."

This promises to be a particularly effective mechanism for overcoming a number of the problems alluded to in the foregoing sections. It is to be hoped that if this program is effective, it will be ongoing.

It is evident that the many suggestions offered in the report will require additional support in order to implement them. It should be also be stated that the magnitude of resources required is, in no way, huge. However, the probable dividends in terms of benefits to humans are enormous, including new products, new jobs, betterment of the environment, along with much expanded basic knowledge of one of the key components of nature.

PART II

AREAS OF SPECIAL OPPORTUNITY

In view of the fact that some aspects of plant biochemistry have been explored in depth, whereas others are relatively underdeveloped, one of the goals of the workshop was to identify those areas that appear to offer the most promise for a significant advance in knowledge. This is a complex issue that can be addressed at many levels. To one scientist, a significant advance implies cloning and characterizing all the genes in a pathway and understanding how the genes are regulated. To another, this may mean obtaining refined measurements of the *in vivo* rates of flux through each of the steps of a pathway and of the reaction mechanisms and kinetic constants for all the enzymes in a pathway. Obviously, these and many other types of information are required in order to form a complete view of any aspect of mechanistic plant biology. However, since the resources that can be devoted to these questions are limited, as are the number of qualified investigators, it is legitimate to ask whether certain areas should be pursued more vigorously or with greater immediacy than others. Is an area more promising because very little is known, and, therefore, the probability for an advance is greatest? Or, are the most promising areas those that are most advanced and, therefore, can be understood in the greatest detail?

In considering these and related issues, several aspects of plant biochemistry appear to offer exceptional opportunities for significant progress for a variety of different reasons. These areas are outlined in the following sections. Although many outstanding problems remain in other areas, there was broad agreement that many of these topics are being adequately addressed by the existing community of scientific expertise and by the currently available mechanisms for providing research support in these areas. For many of these areas no special initiatives appear to be required in order to maintain steady progress. In part this may be due to the fact that some of the areas that were recognized to be most advanced, such as photosynthesis and nitrogen metabolism, have retained priorities in plant biochemistry for several decades and have benefitted from a decade of targeted support by Federal agencies and other institutions throughout the world. However, as an example, there are other inorganic biochemical conversions of ions such as silicon, calcium and numerous others that are still quite lacking in understanding and worthy of study. Clearly, the role of plants in transforming mineral elements has importance in nutrition, in environmental processes and adaptation as well as relationships to plant growth.

Technical Advances

It has been said that "Biochemistry advances on the leading edge of new technologies". Problems that were intractable are resolved by the development of new techniques that frequently arise in adjacent areas. One development that is likely to have a major impact on progress in plant biochemistry is the development of large-scale cDNA sequencing projects.

Although these projects have only recently begun in the U.S. and Europe, it is already evident that this approach will result in identification of many hundreds of new genes by comparison of deduced amino acid sequences to known proteins. In many cases, relatively small regions of conserved sequence in an active site is sufficient to identify the probable function of a gene product. Because of the large amount of sequence information available for enzymes from microbial organisms and animals, this approach will be a very valuable tool for expanding the availability of cloned genes for enzymes that would otherwise be difficult to isolate. For instance, cytochrome P450s are thought to play a very important role in plant metabolism, particularly in the synthesis of many secondary metabolites. Because these enzymes are membrane bound, they have been difficult to characterize by conventional biochemical approaches. However, from the first thousand randomly sequenced *Arabidopsis* cDNA clones, at least five new cytochrome P450 enzymes were identified. The availability of these genes will permit new approaches to the investigation and manipulation of this very important class of enzymes. Similarly, it is expected that many other enzymes will become available by this route. By exploiting new methods for the expression of cloned plant genes in microbial hosts, it is now routinely possible to produce large quantities of enzymes from cloned plant genes. Thus, the properties of these enzymes can be studied *in vitro*, whereas previously this was impossible.

The increasing availability of large quantities of pure proteins has created new opportunities for detailed structural and mechanistic studies of plant proteins. However, there are a relatively small number of laboratories involved in protein chemistry and virtually none of these are devoted entirely to plant proteins. For this reason, the most promising mechanism for fostering the development of this area is to encourage collaborations between plant biologists who have defined and purified interesting proteins, and enzymologists or structural biologists with expertise in protein-NMR, Mossbauer spectrometry, X-ray crystallography and related methods.

Other developments include the proliferation of genes for transcriptional factors, the development of model genetic systems such as *Arabidopsis*, new methods for cloning genes by complementation of microbial mutants, plus others. While the progression of techniques of molecular biology have created exceptional opportunities for advancing plant biochemical research, at the same time the progress in chemical analytical technologies has also opened extraordinary opportunities. Examples of advances in techniques are to be found in mass spectrometry and nuclear magnetic resonance (NMR) spectrometry. These advances permit the rapid elucidation of the structures of newly discovered natural plant products. Likewise, techniques such as X-ray crystallography, have been combined with site-directed mutagenesis to gain more knowledge about how enzymes operate at the molecular level. Thus, there are many new approaches which can be exploited singly, or in combination, to gain a better understanding of fundamental mechanisms. This, in turn, should facilitate the identification and implementation of possible new applications.

Metabolic Engineering

One of the most dynamic and exciting areas of plant biology involves the production of transgenic plants in which the level of activity of an enzyme is increased or decreased by altering expression of the corresponding gene. In many cases the expression of the gene can be decreased by expressing an antisense copy of the gene. Conversely, the amount of activity of an enzyme may be increased by placing the coding sequence behind a strong promoter or, in some cases, by utilizing a gene for an enzyme with altered allosteric properties. The utility of this approach is well illustrated by recent experiments concerning the first enzyme of the starch biosynthetic pathway, adenosine diphosphate glucose pyrophosphorylase. Transgenic potatoes were produced that expressed a bacterial gene encoding a feedback insensitive form of the enzyme. This resulted in a large and commercially significant increase in starch accumulation, thereby conclusively demonstrating the key role of this enzyme in regulating starch accumulation. In a complementary series of experiments, activity of the enzyme was strongly reduced in transgenic potatoes by expression of an antisense version of the mRNA for the enzyme. As a result, the plants produced more potatoes, indicating a link between carbohydrate status and developmental control of tuber formation. In addition, storage protein accumulation was strongly suppressed, indicating a tight link between carbohydrate metabolism and nitrogen metabolism. Nothing is known about the molecular basis of this potentially important regulatory phenomenon.

The first generation of experiments utilizing this approach has revealed that, even in those aspects of metabolism where there is substantial depth of information, it can be difficult to predict the outcome of an experiment involving pathway manipulation. For example, it was thought that the cytosolic enzyme pyrophosphate-dependent phosphofructokinase (PFP) plays an indispensable role in glycolysis. However, transgenic potato and tomato plants that contain reduced levels of PFP (caused by expression of antisense PFP RNA) appear to be normal in their growth patterns and in response to environmental perturbation including inorganic nutrient limitation. This unexpected result highlights the fact that plants are metabolically very flexible and may contain a certain degree of biochemical redundancy. This and a number of similar recent experiments emphasize the importance of being able to apply direct tests of hypotheses which have been developed solely on the basis of physiological and biochemical evidence. It seems likely that this approach that will revolutionize our understanding of plant biochemistry and related areas. Furthermore, because the approach produces unambiguous results, it is expected to revitalize interest in many aspects of biochemistry. As one participant noted:

"Many metabolic pathways that we have become familiar with as a static series of reactions in textbooks are now found to

have interesting mechanisms of regulation at the enzymatic, transcriptional and developmental levels."

The use of molecular genetics to alter metabolism offers many novel opportunities to understand aspects of cellular metabolism about which little is known. It should now be possible to acquire an understanding of the mechanisms that regulate the flux of carbon and nitrogen into the major metabolites. The role of catabolic processes is also poorly defined and needs to be broadly explored. Since many of the genes for the central pathways of carbon and nitrogen metabolism have been cloned, there are now many excellent opportunities to examine the regulation of primary carbon and nitrogen metabolism.

The Cell Wall

The cell walls of higher plants represent the most abundant form of biomass. The chemical composition of the walls determines the structural properties of wood and fibers such as cotton, linen and rayon. Cell walls provide the major component of forage crops and there is abundant evidence that the chemical composition of the walls strongly affects the digestibility of the plant material and, therefore, the feed efficiency. One of the largest industrial uses of cell walls is for paper production, a process that uses large amounts of energy and generates relatively large amounts of waste. Improved knowledge of the structure of the cell wall may permit modifications of the papermaking process so that less chemical waste is generated, less energy is required and so that more of the biomass is utilized. At the biological level, cell walls provide the mechanical stabilization of the plant body. In contrast to animal systems, cell migrations do not contribute to the development of the plant body. The morphology of higher plants is, therefore, solely determined by the planes of cell division within a plant tissue and the subsequent three-dimensional expansion of the individual cells. Thus, detailed knowledge of the plant cell wall may also be essential in understanding the mechanisms responsible for the size and shape of various plants. In view of the large number of uses of plant cell walls, there seems little doubt that the cell wall is an attractive target for modification by genetic engineering. However, there are large gaps in our knowledge of the biochemistry of the cell wall which currently preclude the rational application of biotechnologies to wall modification.

Considerable progress has been made in delineating the primary structures of the quantitatively predominant cell wall components, including pectins, hemicelluloses, cellulose and structural proteins. There has also been substantial progress in determining the structural components of lignin, although many elementary questions about the biosynthesis and degradation of lignin remain (See DOE publication CONF-8804243, a summary report of a workshop on Needs and Opportunities in Lignin Research). For instance, nothing is known about the mechanisms that regulate fluxes into various lignin precursors, the mechanisms involved in export of monolignols across the plasma membrane, or the mechanisms involved in polymerization of lignin precursors.

Similar questions concerning cutin and suberin synthesis are also unsolved. An inherent problem in studying these and related questions is that intact tissues represent a mixture of developmental states. Recent progress in establishing cell cultures in which lignin deposition can be induced by a change in the culture conditions provides important new opportunities to address many fundamental questions in this area. Similarly, there is little or no information about the degree to which the polysaccharide composition of different cell types within a tissue varies. In order to address this and related issues, it will be necessary to develop technologies which would permit structural elucidations of small amounts of material. Additional information is also needed concerning the organization of the individual polymers within cell walls, and the degree to which the various polymers interact via covalent and non-covalent interactions. The development of a comprehensive collection of monoclonal antibodies against cell wall epitopes may be a useful step in this direction. The availability of such probes would permit the application of powerful new cytological methods to the investigation of wall organization. Methods must also be developed to relate such interactions to the structure and function of the wall. What are the roles of the many different kinds of individual polysaccharides and structural proteins?

Relatively little is known about the biosynthesis of cell wall polymers. It is clear, however, that the regulatory network required for the coordinated synthesis of cell wall material in response to cell cycle, cell type, hormonal control of cell elongation, and environmental influences like temperature, wounding, and pathogen attack is tightly controlled. Several hundred genes must be involved in the synthesis of cell walls, their controlled degradation, and the regulation of both processes. Among other things, these genes are expected to encode enzymes for precursor biosynthesis, membrane carriers, glycosyl transferases, glycanases, and enzymes involved in cross-link formations. A small number of laboratories are currently investigating the synthesis of cellulose, xyloglucans, mixed-linked glucans and lignin. However, no researchers appear to be currently investigating the synthesis of the other components. Also, no higher plant cell wall polymerizing enzyme has yet been solubilized and purified or the corresponding gene cloned. This rather surprising fact reflects the difficulties associated with cell wall enzymology, but also highlights exceptional scientific opportunities. Significant progress has been made recently in the solubilization and partial purification of some polysaccharide synthases, elimination of the proposed role of glycolipids as intermediate sugar carriers in polysaccharide synthesis and detailed sub-cellular localization of several of the steps in cell wall polysaccharide biosynthesis. Thus, it is evident that the application of new preparative and analytical methods will permit progress in this area.

Plant cell walls are not static structures, but display a dynamic behavior in response to environmental and hormonal stimuli. After wounding of plant tissues or during pathogen attack, new cell wall material can be rapidly deposited to seal lesions within the affected plant tissue and to retard the growth of invading microorganisms. Some of these and other responses appear to be triggered by cell wall fragments produced by hydrolytic enzymes released by the pathogens. What are the signal molecules involved in such responses and how do they trigger biological responses? During fruit ripening and abscission processes, cell wall polysaccharides undergo controlled degradation by exo- and endoglycanases, and possibly transglycosylases as well as polysaccharide hydrolases, thereby greatly reducing the mechanical strength of the affected wall structures. How is the reduction in mechanical strength controlled during these developmental stages and during the cell cycle? There is evidence that wall-derived oligosaccharide fragments function as morphogenic signal molecules or have a role in modulating hormone action. The cell wall is also thought to be of importance in the interaction of plants with symbionts such as rhizobia and mycorrhiza. What are these signals and how do they act?

There is a pressing need to identify genes encoding enzymes involved in cell wall biosynthesis. These genes can be used to modify cell wall composition in transgenic plants in order to understand the role of the protein and non-protein structural components. The availability of genes would also permit production of large amounts of the enzymes in microbial or plant hosts so that the enzymatic properties can be determined. Genes and antibodies raised against the gene products can be used to study the mechanisms that regulate cell wall biosynthesis. Because of the many uses of plant cells walls, it is to be anticipated that pertinent genes will not only be useful for experimental purposes but may lead directly to useful modifications of the cell wall of various fibre species.

In view of the utility of mutants for dissecting other aspects of biology, it seems likely that a genetic approach will also contribute to dissection of the structure and function of the wall. Several mutants of *Arabidopsis* with altered lignin and polysaccharide composition have recently been described, and mutants of maize are known with phenotypes that suggest alterations in lignin and cell wall composition. The development of facile methods for the identification of additional mutants should prove highly productive.

Biosynthesis of Starch and Lipids

Many of the most promising applications of knowledge of plant biochemistry derive from the fact that higher plants are a very efficient source of renewable organic materials. In particular, corn starch, which is both a useful organic chemical and an important human and animal food, is currently produced in large quantities at about the same cost per pound as crude petroleum. Although the uses of corn starch as a chemical feedstock are currently limited by its molecular composition, it is possible to envision that with genetic modifications, a broad range of structurally modified starches and other useful compounds could be produced from maize in high volume and at low cost. Many of the genes involved in starch biosynthesis have recently been cloned and it is, therefore, now possible to explore the possibility of creating new kinds of starches for use in non-food applications by changing the degree of branching or other modifications of starch structure. For instance, by creating new kinds of starch, it may be possible to expand the use of starch as a co-polymer in new biodegradable plastics that contain as much as 80% starch. The increased used of starch and other biopolymers for non-food applications would reduce our dependence on the use of petroleum and permit the increased use of renewable and environmentally benign materials. Recent progress in producing biodegradable plastics in genetically engineered plants may also be seen as a small step in this direction.

Plant storage lipids are also produced in very large quantities and represent a chemically versatile form of biomass. Tropical plants such as oil palm are very efficient producers of oil. Yields of up to 14,000 pounds of oil per hectare per year have been obtained from this species. Progress toward increasing the oil yield and oil composition of this and other important oil-producing species will depend upon understanding the factors that regulate oil biosynthesis. These are thought to include regulatory mechanisms operating at the level of the triacylglycerol biosynthetic pathway, developmental mechanisms that regulate the formation of storage tissues, and the mechanisms that regulate carbon translocation. Knowledge of these factors must be based on a foundation of detailed information about the underlying metabolic pathways. The major metabolic pathways leading to lipid synthesis in plants have been defined primarily at the level of radioisotope tracer studies. However, in the past several years several enzymes involved in plant lipid metabolism have been purified to homogeneity and a dozen or more genes involved in the early reactions of fatty acid and lipid synthesis have been cloned. There is also a substantial amount of genetic diversity in plant oil composition which has been useful in defining the biosynthetic pathways and in permitting the cloning of several genes by genetic methods. This area is developing very rapidly and several modifications of plant oils by genetic engineering have been reported.

Although a narrow range of fatty acids is found in field crops, at least 200 different kinds of fatty acids are found in the lipids that are accumulated as storage reserves by various wild species. In general, plant fatty acids can have double or triple bonds at many positions along the hydrocarbon chain and these unsaturations may be accompanied or replaced by various substituents such as cyclopropenyl, hydroxyl, furan, or epoxy groups. Many of these unusual fatty acids could be of substantial industrial use for fuels, lubricants and other usages if they could be produced in comparable quantities and with the same efficiency as edible oils are currently produced in field crops. It seems likely that this can be accomplished by transferring genes for one or a few fatty acid modifying enzymes from undomesticated species to crop species. However, the base of biochemical knowledge about the enzymes involved is very thin. No fatty acid modifying enzyme has been purified, nor any gene for a modifying enzyme cloned from a plant to date. In some cases, this is quite surprising. For instance, ricinoleic acid, the main constituent of castor oil, is among the world's most versatile natural products and has many dozens of industrial uses that includes the synthesis of nylon-11, lubricants, hydraulic fluids, plastics, cosmetics, and other materials. Because of several important uses, ricinoleic acid is considered a strategic material by an act of Congress. Approximately $300,000,000 worth of this material is used each year worldwide. However, very little is known about the mechanisms by which this fatty acid is synthesized and stored by the castor plant, and during the twenty year period from 1972 to 1992, there was only one scientific publication concerning the biosynthesis of this compound.

Starch and lipids are accumulated in nonphotosynthetic tissues where their synthesis is dependent upon the availability of fixed carbon and nitrogen from leaves. The engineering of plants for production of novelty starches, lipids and other compounds will therefore require knowledge of the biochemistry of developing storage tissues and the developmental processes required for their formation. Basic knowledge of these factors may lead to novel strategies to augment overall productivity by increasing the number of storage organs per plant or by prolonging the synthetic phase of the desired macromolecule or specialty chemical. The transport of metabolites and partitioning of carbon and nitrogen among organelles is a central part of metabolic biochemistry that is poorly understood. For instance, very little is known about the proteins that mediate the loading of sucrose into or out of the phloem during transport throughout the plant. As one participant noted:

"Identification of the proteins involved in carbon transport, and in the regulation of source-sink relations, would enhance our ability to divert carbon to appropriate organs and increase the amounts of starch and oils in tubers and roots."

The partitioning of pathways among organelles at the cellular level also poses many relevant problems. The individual transporters and what they transport are not well characterized, particularly in non-photosynthetic tissues. For instance, we do not know what intermediates are transported into plastids to support fatty acid biosynthesis. Similarly, there is still uncertainty about the metabolites which are transported into amyloplasts to support starch synthesis. Lack of knowledge about these fundamental aspects of cellular metabolism poses a strong impediment to attempts to divert carbon into novel compounds. Thus, it is essential to develop a detailed understanding of the mechanisms which regulate flux of carbon and nitrogen from one cellular compartment to another and from one tissue to another.

Terpenoids

There are currently more than 20,000 terpenoid structures reported, the majority of which are from higher plants. Many of these that have value as medicinals, polymers (e.g. rubber), solvents, flavorings, fragrances or coloring agents. Certain terpenoids have also been implicated as antimicrobial agents and as regulators of insect behavior or development. In spite of the prominence of these compounds in the plant kingdom, relatively little work has been undertaken, as yet, on the isolation of genes for terpene biosynthetic enzymes. Many of these are thought to be membrane bound and are, therefore, difficult to purify. Few laboratories in the world are actively engaged in the enzymology of plant terpenoids. In view of the importance of this diverse family of natural products, progress in their enzymology was considered a priority.

An overwhelming problem with terpenoids is that there are so many different representatives. It is not possible to characterize all the pathways. Fortunately, in spite of the large number of chemical structures, it is estimated that a family of only a few hundred enzymes may be required to convert the ubiquitous precursors to representative members of the major classes of terpenoids. Furthermore, many of these enzymes may be structurally related enzymes with slightly different specificities such as has been observed for cytochrome P450s in animals, and fatty acid desaturases in plants. Thus, it seems likely that if a gene for a key enzyme, such as a sesquiterpene cyclase, were available, this gene could be used as a heterologous probe to identify genes for structurally related but catalytically heterologous enzymes from a large number of species. Thus, a systematic effort should be undertaken to isolate a gene for each representative enzyme catalyzing the principal reactions involved in the synthesis of the major classes of terpenoids.

Another priority for this area is to obtain information on the mechanisms that regulate flux through the pathway and into the various types of terpenoid products. Several classical biochemical approaches to this problem are applicable. Additionally, the availability of genes for an entire pathway in an easily transformed organism would greatly facilitate a detailed analysis of this problem using molecular techniques once putative rate-limiting steps have been identified. Given the tremendous diversity of the terpenoid pathway, the selection of several "model species" for detailed metabolic and molecular studies would be particularly useful. Tomato was suggested as a possible model system because of the excellent genetic resources available in this species.

Many terpenoids are accumulated in glands, ducts, trichomes, lactifiers or other specialized structures. Thus, attempts to enhance the level of accumulation of economically important terpenoids, such as taxol, may also depend upon knowledge of the mechanisms that regulate the proliferation of these special structures. The development of a suitable genetic system would also greatly facilitate progress on this aspect of the general problem.

Cell-Specific Metabolism, Biochemical Adaptation, and Secondary Metabolism

Higher plants collectively synthesize an enormous spectrum of chemical constituents which are generally considered to be secondary metabolites because they do not appear to play an essential role in the growth and development of the plant. Many secondary metabolites have medicinal value or other economic uses, many are important in plant defense mechanisms or in abiotic stress responses, and some confer important horticultural qualities to plants. The biosynthesis of certain classes of these compounds, such as anthocyanins, or certain alkaloids such as nicotine or the *Vinca* alkaloids, have been studied in great detail. Other classes of compounds, such as most alkaloids and di- and tri-terpenes, have received comparatively little attention. Because of the chemical diversity of secondary metabolites, it is necessary to identify an efficient mechanism to obtain biochemical information about this class of compounds that would complement the extensive ongoing research concerning the identification and structural elucidation of new compounds.

One approach to this issue arises from a consideration of the origin of chemical diversity in plants. The angiosperms are thought to have evolved during the last 150 - 200 million years. Thus, the chemical diversity of secondary metabolism represents the result of a fascinating process of evolution of new enzymes. By extrapolation from studies of animal and microbial systems, it is apparent that many enzymatic reactions associated with secondary metabolism are catalyzed by structurally related enzymes that have evolved slightly different substrate specificities. Furthermore, some of the enzymes involved in secondary metabolism have broad substrate specificity so that the specificity of a particular reaction is controlled by the availability of substrate. One implication of these principles is that it may prove rewarding to complete a detailed characterization of a representative enzyme from each major family of related enzymes involved in the synthesis of secondary metabolites. As structural information about these enzymes accumulates, it will become possible to use this information to isolate genes for enzymes from diverse species that catalyze similar reactions. Thus, careful consideration should be given to elucidating the series of reactions that lead to the formation of particular compounds, and the purification of relevant enzymes.

There are many instances in which it would be desirable to increase the amount of accumulation of a secondary metabolite. A notable example is the current necessity of processing 750,000 lb of bark from *Taxus brevifolia* to produce 50 lb of the anticancer diterpene taxol. In some instances increased expression of a single enzyme is sufficient to cause increased accumulation of a secondary metabolite. For example, *Atropa belladonna*, normally accumulates high levels of the anticholinergic tropane alkaloid, hyoscyamine, but has low levels of the more valuable alkaloid scopolamine. Introduction of a constitutively expressed gene for hyoscyamine 6β-hydroxylase from *Hyoscyamus niger* in transgenic *A. belladonna* plants resulted in plants that accumulated high levels of scopolamine in their leaves. Such examples notwithstanding, the essential problem in most cases is that the compounds of interest are produced by a multienzyme pathway in which it is probably necessary to increase the amount of activity of many enzymes in order to effect increased flux through the pathway. The results of several recent experiments suggest that in at least some multienzyme pathways, all the enzymes of the pathway are under transcriptional control of a small number of *trans*-acting factors. This raises the possibility of producing transgenic plants in which overexpression of a transcriptional factor causes a corresponding increase in the level of expression of the enzymes of a complex pathway. An example of this principle was the recent demonstration of the production of high levels of anthocyanins in various tissues of maize by the introduction of a gene for the *Lc*-transcriptional factor that was placed under control of a constitutive promoter. Constitutive expression of this transcriptional factor resulted in the accumulation of high levels of anthocyanins in tissues, such as roots, which do not normally accumulate pigments. Thus, by analogy, it seems likely that the isolation of *trans*-acting factors that regulate accumulation of other classes of secondary metabolites may have broad utility in increasing accumulation of related metabolites in heterologous species. There are now several powerful new approaches to the isolation of such transcriptional factors. However, the application of these and related methods for enhanced production has been impeded by the dearth of biochemical information available.

These examples illustrate the encouraging fact that there is the possibility for a strongly synergistic relationship between the extensive research concerning mechanisms of gene regulation, and the research concerning mechanisms that regulate the accumulation of secondary metabolites. Mechanisms that would encourage collaboration between researchers concerned with gene regulation and those concerned with understanding the regulation of secondary metabolism are likely to be very productive. Furthermore, many secondary metabolites are thought to be involved in disease and pest resistance or in stress tolerance. Accordingly, the accumulation of these compounds is frequently strongly stimulated by exposure of a plant to biotic or abiotic stress. There are a large number of intriguing unanswered questions concerning the mechanisms by which the biosynthetic pathways are induced and the role of the secondary metabolites in the stress response. For example, it is not known whether different plant species use similar signal transduction pathways to induce expression of different secondary metabolites in response to a particular stress. The answer to this and related questions could have broad academic and applied significance for understanding adaptive mechanisms in plants.

Perception, Response and Adaptation to the Environment

Plants are normally exposed to a wide range of environmental conditions which may include exposure to biotic as well as abiotic stresses. The analysis of the various mechanisms involved in the perception, response and adaptation to environmental changes requires a broad spectrum of approaches which include biochemical analyses. Although there is substantial activity in this area, which is generally pursued under the rubric of signal transduction, much of this work is focused on mechanisms of gene expression. One participant expressed the view, which was echoed by several others:

"Studies of signal transduction in plants

are currently too narrowly focused on gene expression. There is a pressing need to understand the underlying biochemical mechanisms. However, this problem seems to have fallen between the cracks because biochemists think it is cell biology and cell biologists think it is biochemistry. Perhaps this is an area where we need mechanisms that will encourage interactions between individuals with different expertise."

In extending this discussion another participant noted:

"There is a need to make the connection between small molecule biochemistry, and cell biology. Plants have developed different small second messengers than animals and this needs to be explored by scientists who are knowledgeable about analytical methods."

As an example of an underdeveloped area of potentially great importance it was noted that, as in animals, lipids have been implicated as signal molecules in plants; recent work has implicated inositol lipids and jasmonic acid in signal transduction pathways. However, almost nothing is known about the pathways of inositol lipid synthesis and degradation in plants, and there is very little current activity in this area. This is an area which is badly in need of more intensive investigation. On the other hand, the recent discovery of the role of jasmonic acid as a signal molecule in plants also emphasizes the fact that plants have acquired completely novel signal molecules. This is also evident from recent work on the elucidation of nodulation factors and from evidence implicating cell wall fragments as elicitors. These discoveries are likely to stimulate the search for additional signal molecules and suggest that this aspect of plant biology is in comparatively good health.

The other aspect of the general problem of environmental response concerns the elucidation of the changes in cellular metabolism and cell structure. It has become increasingly apparent that adaptation to biotic stresses or abiotic stresses caused by extremes of temperature or soil water content, high salt, metals and other factors results in changes in cell composition and metabolism. The increased availability of sensitive analytical techniques for structural determination should facilitate the elucidation of the biochemical changes which occur in response to various stresses. Also, it is now possible to directly test the role of such changes by the production of transgenic plants or mutants which have altered capability to undertake such changes. For instance, membrane lipid composition is thought to be important in the ability of higher plants to survive temperature stress. Because many of the same enzymes participate in both membrane and storage lipid synthesis, the concurrent progress in developing genetic methods to modify storage oil composition has converged with experiments designed to test the role of fatty acid unsaturation in temperature acclimation. Similarly, progress in defining the details of carbohydrate metabolism has permitted the production of transgenic plants with increased salt tolerance due to the accumulation of increased levels of sugar alcohols. Because many of the stresses experienced by plants are not problems for animals, it seems that this area may produce novel biological principles. Also, since various stresses are generally considered to be the major limitations to plant productivity, improved understanding of the underlying biochemistry may afford new opportunities for rational plant improvement.

Appendix I

NAMES AND AFFILIATIONS OF PARTICIPANTS RESEARCH OPPORTUNITIES IN PLANT BIOCHEMISTRY WORKSHOP DECEMBER 11-13, 1992, KONA, HAWAII

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Appendix II

WORKSHOP ON OPPORTUNITIES IN PLANT BIOCHEMISTRY
SUGGESTED LIST OF DISCUSSION TOPICS

1. What is the level of knowledge of key metabolic pathways; what are the ill-defined reactions of the pathways and how valid are the commonly accepted pathways?

2. What are the regulatory mechanisms governing the flux through key metabolic pathways?

3. How prevalent is the existence of alternate or redundant pathways, and what are the determinants of flux into one or another of the pathways?

4. What are the roles of cellular structures, tissue differentiation, and temporal development in regulating metabolism?

5. What are the contemporary issues pertaining to assimilatory processes and to the biosynthesis and catabolism of lipids, carbohydrates, polysaccharides, proteins, phenolics, terpenoids, and secondary metabolites?

6. Is it possible to identify uniquely favorable organisms or experimental approaches for the study of the various metabolic pathways? Are there new research technologies that offer novel opportunities for rapid progress?

7. The diversity of plant natural products is enormous as are the associated metabolic pathways. On the other hand, research resources are limited. How should the priorities for research support be established? What criteria should be used?

8. What are the human resources supporting research activities in plant biochemistry? What mechanisms would be effective in stimulating increased research in this area? What are the career opportunities for young investigators?

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