MASTER

PLANT RESEARCH '76
MSU-ERDA Plant Research Laboratory

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Cover: Promotion and inhibition of flowering in a dayneutral plant, the tobacco cultivar Trapezond, studied in grafting experiments with the short-day plant Maryland Mammoth tobacco and the long-day plants *Nicotiana glauca* and *Hyoscyamus niger*. In all cases, left-hand graft of a pair on long days, right-hand graft on short days; in each graft, the 'indicator shoot' of Trapezond (i.e. the shoot on which the flower response is registered) on the left, the scion whose influence on the flowering response of Trapezond is measured, on the right.

Upper left: control grafts, Trapezond on Trapezond--little effect of daylength on flower formation in the indicator shoot. (Flowers on the scion were removed as they appeared.)

Upper right: grafts Maryland Mammoth on Trapezond--little effect on flower formation in the dayneutral plant by a non-flowering short-day plant; promotion by a flowering one.

Lower left: grafts *N. glauca* on Trapezond--promotion of flower formation in the dayneutral plant by a flowering long-day plant, strong inhibition by a non-flowering one.

Lower right: grafts *H. niger* on Trapezond--same results as in grafts with *N. glauca*.

For details, see p. 23-27

PLANT RESEARCH '76

Report of the
MICHIGAN STATE UNIVERSITY-ENERGY RESEARCH
AND DEVELOPMENT ADMINISTRATION
PLANT RESEARCH LABORATORY

NOTICE

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for the Year 1976

MSU-ERDA Plant Research Laboratory

Michigan State University

East Lansing, Michigan 48824, USA

1976

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DIRECTOR'S REPORT

Research

1976 has been the year in which, while the other tasks continued their programs, the two tasks whose scope had been expanded--K. Raschke's on plant-environment interrelations as regulated by the stomatal apparatus, and C. P. Wolk's on nitrogen fixation and development in blue-green algae--and the two that had been formed "de novo"--one on plant resistance to environmental stress, particularly water stress (A. D. Hanson), and one on sulfur dioxide injury and resistance in plants (P. Filner with L. G. Wilson)--have settled down to meet their objectives. However, some of their activities have already been described in last year's Director's Report, preempting work that was actually not accomplished or completed before 1976.¹ There would be no point in repeating these accounts, and we will also forego other details of the research carried out in 1976, referring the reader to the individual research reports. The overall objective of the PRL research program remains unchanged--to contribute to the knowledge, with strong emphasis on fundamental problems, of how plants function, the roles they play in the environment and the energy relations of the world, and how these roles may be optimized for the benefit of mankind.

New faculty

Andrew D. Hanson was appointed Assistant Professor effective July 1, 1976. Before this appointment, Dr. Hanson has already been with the PRL for not quite 3 years, first as Research Associate with H. J. Kende, working mainly on ethylene synthesis and action in aging Morning glory (*Ipomoea*) flowers, and since July 1, 1975 as Specialist, initiating--under the general supervision of the Director--the task on resistance

¹It seems, however, that our math, at least that with Roman numerals, was substandard when that report was prepared. A "V" was left out at the top of page 7, and the "IV" on p. 8 should have been a "VI".

of plants to environmental stress. He is a native of the U.K. and received his B.S. and Ph.D. degrees from Queen Elizabeth College, University of London, under J. Edelman. His Ph.D. work dealt with carbohydrate metabolism in carrot and sugar cane tissue cultures. After receiving the Ph.D. in 1971, Dr. Hanson worked for a year (1971-72) in the Crop Science Department of Rank, Hovis, McDougall Research, Ltd., under Dr. D. A. Doling, mainly on the problem of "vigor" in cereal seeds. He was subsequently awarded a Fellowship of the Royal Society, to work at the Centre Universitaire de Marseille-Luminy, France, on RNA metabolism in hormone dependent and independent plant cell suspension cultures (1972-73), and then came to the PRL in November 1973.

Teaching

The following faculty members participated on a regular basis in instruction (scheduled courses, excluding research):

Deborah Delmer

Biochemistry 200. Introduction of Biochemistry (5 credits)**

Biochemistry 855. Plant Biochemistry (4 credits)**

Philip Filner

Human Medicine 511. Phase II, Term 1, Focal Problems (4 credits)**

Alfred Haug

Biophysics 402. Introductory Biophysics (3 credits)***

Biophysics 821. Molecular Biophysics (5 credits)*

Hans Kende and Jan Zeevaart

Botany 415. Plant Physiology: Growth and Development (5 credits)

Kenneth Poff and Jan Zeevaart

Botany 856. Advanced Plant Physiology: Growth and Development (3 credits)

Peter Wolk

Botany 841. Physiology of Algae (3 credits)

* Jointly with another instructor

** Jointly with two other instructors

*** Jointly with several other instructors

Training

The following graduate students (graduate assistants) working in the Laboratory obtained their degrees:

<u>Name</u>	<u>Present location and position</u>
(in parentheses, thesis supervisor and MSU Department conferring degree)	
Jean-Cheui Hsung, Ph.D. (A. Haug, Biophysics)	Department of Chemistry State University of New York At Albany Albany, New York, Research Associate
Michael Sussman, Ph.D. (H. Kende, Botany & Plant Pathology)	Biology Department Yale University New Haven, Connecticut Research Associate

The following research associates and specialists completed their periods of work and training in the Laboratory; * indicates an investigator who was on leave from and has returned to his home institution, and who had his personal support:

<u>Name</u>	<u>Present location and position</u>
(in parentheses, supervising faculty member)	
Peter Beutelmann (H. Kende)	Institut für Allgemeine Botanik, University Mainz, West Germany, Research Fellow
Mary Ericson (D. Delmer)	Department of Biochemistry University of Texas Health Science Center San Antonio, Texas, Research Associate
Graham Farquhar (specialist) (K. Raschke)	Department of Environmental Biology Research School of Biological Sciences Australian National University Canberra City, Australia, Research Fellow
Stuart Flashman (P. Filner)	Department of Genetics North Carolina State University Raleigh, North Carolina, Assistant Professor

<u>Name</u>	<u>Present location and position</u>
(in parentheses, supervising faculty member)	
Gary Gardner (H. Kende)	Shell Development Company Modesto, California, Research Scientist
Katsushi Manabe (K. Poff)	Department of Biological Sciences University of Illinois at Chicago Circle Chicago, Illinois, Research Associate
Werner Schmidt (K. Poff)	Fachbereich Biologie Universität Konstanz, West Germany, Research Fellow
Joseph Thomas* (P. Wolk)	Biology and Agriculture Division Bhabha Atomic Research Centre Trombay, Bombay, India Scientific Officer,
Ludo Van Rompuy (J.A.D. Zeevaart)	Lab. voor Fysiologische Chemie Ledeganckstraat 35, B-9000 Gent Belgium, Researcher

Faculty activities and awards

D.T.A. Lamport was awarded a John Simon Guggenheim Foundation Fellowship for a sabbatical year at the John Innes Horticultural Institution, Norwich, United Kingdom (started October 1, 1976). This is the sixth such award to a faculty member of the PRL.

A. Lang returned from a sabbatical leave spent at the K. A. Timiryazev Institute of Plant Physiology, Academy of Sciences of USSR and supported under the Scientific Exchange Program between the National Academy of Sciences of USA and the Academy of Sciences of USSR (February 17, 1976). He received a Distinguished Faculty Award from MSU in February, and was awarded the Charles Reed Barnes Life Membership and the Stephen Hales Prize of the American Society of Plant Physiologists at its Annual Meeting in New Orleans in June--the first time the Society gave both awards to the same person at the same time. He was also appointed, in September, by President Ford to the President's Committee on the National Medal of Science.

G. P. Wolk was appointed member of the Editorial Board of the Journal of Phycology.

PERSONNEL

Faculty, Including Joint Appointments

Deborah P. Delmer, Assistant Professor of Biochemistry
 Philip Filner, Professor of Biochemistry
 Andrew Hanson, Assistant Professor (Research) From July 1, 1976
 Alfred Haug, Professor of Biophysics
 Michael Jost, Associate Professor of Botany and Plant Pathology
 Hans J. Kende, Professor of Botany and Plant Pathology
 Derek T. A. Lamport¹, Professor (Research)
 Anton Lang², Director and Professor of Botany and Plant Pathology
 Kenneth L. Poff, Assistant Professor of Botany and Plant Pathology
 Klaus Raschke, Professor of Botany and Plant Pathology
 Lloyd G. Wilson, Associate Professor of Botany and Plant Pathology
 (Research)
 C. Peter Wolk, Professor of Botany and Plant Pathology
 Jan A. D. Zeevaart, Professor of Botany and Plant Pathology

Research Specialists (including supervising faculty member)

Graham Farquhar (K. Raschke) Until June 30, 1976
 Andrew Hanson (A. Lang) Until June 30, 1976

Research Associates (including supervising faculty member)

Peter Beutelmann³ (H. Kende) Until August 31, 1976
 Ray Bressan (P. Filner) From January 1, 1976
 Joyce Clarke (D.T.A. Lamport) From March 16, 1976
 Thomas Currier (P. Wolk) From January 16, 1976
 Mary Ericson (D. Delmer) Until December 31, 1976
 Stuart Flashman (P. Filner) Until December 31, 1976
 Gary Gardner⁴ (H. Kende) Until October 14, 1976

¹ On sabbatical leave from October 1, 1976

² On sabbatical leave until February 20, 1976

³ Supported by Max Kade Foundation

⁴ Partially supported by a grant from the National Science Foundation
 to H. Kende

John Haury (P. Wolk) From January 16, 1976
 Wolfgang Lockau (P. Wolk) From June 1, 1976
 Robert Locy (H. Kende) From September 1, 1976
 Katsushi Manabe (K. Poff) Until August 31, 1976
 John Meeks (P. Wolk)
 Richard Peterson (P. Wolk) From December 1, 1976
 Harry Phillips (H. Kende)
 Hartmut Quader¹ (P. Filner)
 Robert Saftner (K. Raschke)
 Werner Schmidt¹ (K. Poff) Until July 14, 1976
 Joseph Thomas² (P. Wolk) Until March 17, 1976
 Raymond Tully (A. Hanson) From December 1, 1976
 Ludo Van Rompuy (J. A. D. Zeevaart) Until December 31, 1976

Graduate Assistants (including departmental affiliation and supervising faculty member)

Donna Bernardoni (Biophysics) - A. Haug, From December 16, 1976
 Kent Bradford³ (Horticulture) - H. Kende and D. R. Dilley
 (Department of Horticulture)
 *Charles Caldwell (Botany and Plant Pathology)
 Judith Cherniack (Biochemistry) - P. Filner
 Dean Dubbe (Botany and Plant Pathology) - K. Raschke, Until
 December 16, 1976
 Takayasu Hiroswa (Botany and Plant Pathology) - P. Wolk
 Jean-Cheui Hsung (Biophysics) - A. Haug, Until December 15, 1976
 Chausing Jack Jen (Biophysics) - A. Haug, From June 16, 1976
 David Kavon (Botany and Plant Pathology) - H. Kende
 Cynthia Kennedy (Botany and Plant Pathology) - J. A. D. Zeevaart

¹ Supported by Deutsche Forschungsgemeinschaft (DFG)

² Partially supported by a grant from the National Science Foundation to
 C. P. Wolk

³ Partially supported by a predoctoral fellowship from the National
 Science Foundation

* Has not yet chosen a major advisor

Anita Klein (Biochemistry) - D. Delmer

Kenneth Leonards (Botany and Plant Pathology) - A. Haug and

E. Cantino (Department of Botany and Plant Pathology)

David Maltby¹ (Biochemistry) - D. Delmer

James Metzger (Botany and Plant Pathology) - J. A. D. Zeevaart

Andrew Mort (Biochemistry) - D. T. A. Lamport

Margaret Pierce (Botany and Plant Pathology) - K. Raschke

*Louis Ptak (Botany and Plant Pathology) Until May 31, 1976

Shirley Rodaway (Botany and Plant Pathology) - H. Kende

*Thomas Sharkey (Botany and Plant Pathology)

Thomas Skokut (Botany and Plant Pathology) - P. Filner, P. Wolk

Michael Sussman (Botany and Plant Pathology) - H. Kende, Until

December 15, 1976

Jeffrey Suttle (Botany and Plant Pathology) - H. Kende

*Philip Trinity (Biochemistry)

Carol Van Kirk (Botany and Plant Pathology) - K. Raschke

*Richard Vierstra (Botany and Plant Pathology) - From September 16, 1976

*Catherine Wasmann (Botany and Plant Pathology) - From September 16, 1976

Herman Weller² (Biophysics) - A. Haug, Until September 15, 1976

Bruce Whitaker (Botany and Plant Pathology) - K. Poff

Narendra Singh Yadav (Botany and Plant Pathology) - P. Filner, H. Kende

Li Lillian Yang (Biophysics) - A. Haug

Technical Personnel (including supervising faculty member; if none listed -- general service)

Susan Atkins, Senior Technician (P. Filner) Until February 27, 1976

Michael Caughey, Senior Technician (D. T. A. Lamport)

Charles Cetas, Senior Technician (J. A. D. Zeevaart)

¹ Supported by a training grant from the National Institutes of Health to the Department of Biochemistry

² Supported by a training grant from the National Institutes of Health to the Department of Biophysics

* Has not yet chosen a major advisor

Alma Clark, Senior Laboratory Assistant
 Peggy Coffman, Senior Technician (P. Filner) From September 13, 1976
 Renate de Zacks, Principal Technician (H. Kende)
 Robert Geyer, Instrument Maker and Shop Supervisor
 Marina Kao, Senior Technician (K. Raschke) Until April 9, 1976
 Robert Kever, Equipment Technician
 Carl Kulow, Senior Technician (D. Delmer)
 Lloyd Le Cureux, Senior Technician (P. Filner) From October 12, 1976
 Elliot Light, Supervisor Research
 Charles Nelsen, Senior Technician (A. Hanson)
 David Nettleton, Senior Technician (P. Wolk) From July 6, 1976
 Dennis Phillips, Senior Technician (K. Poff) From June 16, 1976
 Cheri Schmuck, Senior Technician (K. Raschke) From June 7, 1976
 Paul Shaffer, Senior Technician (P. Wolk)
 Thomas Sharkey, Senior Technician (K. Raschke) Until September 15, 1976
 Michiyo Skokut, Senior Technician (K. Poff)
 Gary Smith, Senior Technician (A. Haug) Until September 15, 1976
 Anna Summers, Senior Laboratory Assistant
 Henry Wieferich, Mechanical Technician

Administrative and Clerical Personnel

Deborah Bruchmann, Clerk Typist II, From January 19, 1976
 Karen Cline, Secretary II
 Homer Curtis, Administrative Assistant
 Nancy Hanson, Secretary I, From September 23, 1976
 Kathryn Harmon, Accounting Clerk II
 Tawn Jarvis, Senior Clerk Stenographer, Until January 19, 1976

PUBLICATIONS

- 1) Cardemil, L., and C. P. Wolk: The polysaccharides from heterocyst and spore envelopes of a blue-green alga. J. Biol. Chem. 251:2967-2975 (1976).
- 2) Fisher, R. and C. P. Wolk: Substance stimulating the differentiation of spores of the blue-green alga *Cylindrospermum licheniforme*. Nature 259:394-395 (1976).
- 3) Hanson, A. D. and H. Kende: Biosynthesis of wound ethylene in Morning-glory flower tissue. Plant Physiol. 57:538-541 (1976).
- 4) Hanson, A. D. and H. Kende: Methionine metabolism and ethylene biosynthesis in senescent flower tissue of Morning-glory. Plant Physiol. 57:528-537 (1976).
- 5) Ho, D. T.-H. and J. E. Varner: Response of barley aleurone layers to abscisic acid. Plant Physiol. 57:175-178 (1976).
- *6) Kende, H.: Ethylene-induced ethylene biosynthesis: A model for a positive feedback mechanism. Nova Acta Leopoldina 7:165-174 (1976).
- 7) Kende, H. and G. Gardner: Hormone binding in plants. Ann. Rev. Plant Physiol. 27:267-290 (1976).
- 8) Kende, H. and A. D. Hanson: Relationship between ethylene evolution and senescence in Morning-glory flower tissue. Plant Physiol. 57:523-527 (1976).
- 9) Porter, J. and M. Jost: Physiological effects of the presence and absence of gas vacuoles in the blue-green alga, *Microcystis aeruginosa* Kuetz. emend. Elenkin. Arch. Microbiol. 110:225-231 (1976).
- *10) Raschke, K.: The stomatal feedback system: responses to carbon dioxide and abscisic acid. (In Russ.) Physiol. Biochem. of Cultivated Plants 8:242-245 (1976).
- *11) Raschke, K.: Transfer of ions and products of photosynthesis to guard cells. In: Transport and Transfer Processes in Plants, J. B. Passioura and I. F. Wardlaw, eds., Acad. Press, New York, Chapter 19, pp. 203-215 (1976).
- *12) Raschke, K.: How stomata resolve the dilemma of opposing priorities. Phil. Trans. Roy. Soc. London B 273:551-560 (1976).

- 13) Raschke, K., M. Pierce and C. C. Popiela: Absciscic acid content and stomatal sensitivity to CO₂ in leaves of *Xanthium strumarium* L. after pretreatments in warm and cold growth chambers. Plant Physiol. 57:115-121 (1976).
- 14) Raschke, K. and J. A. D. Zeevaart: Absciscic acid content, transpiration, and stomatal conductance as related to leaf age in plants of *Xanthium strumarium* L. Plant Physiol. 58:169-174 (1976).
- 15) Reuveny, Z. and P. Filner: A new assay for ATP sulfurylase based on differential solubility of the sodium salts of adenosine 5'-phosphosulfate and -sulfate. Anal. Biochem. 75:410-428 (1976).
- 16) Simon, R. D. and P. Weathers: Determination of the structure of the novel polypeptide containing aspartic acid and arginine which is found in cyanobacteria. Biochim. Biophys. Acta 420:165-176 (1976).
- 17) Sussman, M. R. and R. Firn: The synthesis of a radioactive cytokinin with high specific activity. Phytochemistry 15:153-155 (1976).
- * 18) Varner, J. E. and D. T.-H. Ho: The role of hormones in the integration of seedling growth. In: The Molecular Biology of Hormone Action (34th Symp. Soc. for Develop. Biol), J. Papaconstantinou, ed., Acad. Press, New York-San Francisco-London, pp. 173-194 (1976).
- 19) Wilson, L. and D. Bierer: The formation of exchangeable sulphite from adenosine 3'-phosphate 5'-sulphatophosphate in yeast. Biochem. J. 158:255-270 (1976).
- 20) Wolk, C. P. and P. W. Shaffer: Heterotrophic micro- and macrocultures of a nitrogen-fixing cyanobacterium. Arch. Microbiol. 110: 145-147 (1976).
- 21) Wolk, C. P., J. Thomas, P. W. Shaffer, S. M. Austin and A. Galonsky: Pathway of nitrogen metabolism after fixation of ¹³N-labeled nitrogen gas by the cyanobacterium, *Anabaena cylindrica*. J. Biol. Chem. 251:5027-5034 (1976).
- 22) Zeevaart, J. A. D.: Physiology of flower formation. Ann. Rev. Plant Physiol. 27:321-348 (1976).

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MEETINGS, SEMINARS, LECTURES

Annual Meeting of the American Society of Plant Physiologists and American Institute of Biological Sciences, New Orleans, Louisiana (May-June 1976): Papers were given by P. Beutelmann on "The effect of senescence and ethylene on membrane-lipid levels in excised flower tissue of *Ipomoea tricolor*" (co-authored with H. Kende); D. P. Delmer on "Glucan synthesis from UDP-glucose in cotton fibers" (co-authored with U. Heiniger and C. Kulow); M. C. Ericson on "Biosynthesis of glycoproteins in plants: the role of lipid intermediates" (co-authored with D. P. Delmer); A. D. Hanson on "Biosynthesis of stress-induced ethylene in morning glory flower tissue"; M. Meinert on "Biochemical changes in cell wall composition associated with in vivo and in vitro fiber development in *Gossypium hirsutum*" (co-authored with D. P. Delmer); A. Mort on "Specific deglycosylation via anhydrous hydrogen fluoride" (co-authored with D. T. A. Lamport); K. L. Poff on "Photoreceptor blues; quest for the photoreceptor pigment"; K. Raschke on "Metabolism of malic acid in the epidermis of leaves"; S. J. Rodaway on "Effects of 5-fluorouracil on thermal stability of α -amylase from barley half-seeds"; Werner Schmidt on "Inhibitors of phototropism in corn seedlings" (co-authored with K. L. Poff and P. Filner); L. Van Rompuy on "Analysis of the estrogen-like fraction in higher plants" (co-authored with J. A. D. Zeevaart); D. P. Delmer also chaired a session on auxins and cell walls, and K. L. Poff one on "Photomorphogenesis: circadian rhythms".

Annual Meeting of the Midwest Section of the American Society of Plant Physiologists, Michigan State University, East Lansing, Michigan, (August 1976): Papers were given by D. Bell on "Effects of uncouplers of photophosphorylation on the luminescence of chloroplasts, measured microseconds after illumination" (co-authored with N. Good and A. Haug); G. Gardner on "In vivo uptake and binding of ^3H -benzyladenine in moss protonemata" (co-authored with H. Kende and R. de Zacks); A. D. Hanson on "Proline accumulation and water potential in the leaves of two barley cultivars of differing drought-resistance" (co-authored with C. Nelsen); K. Manabe on "Blue light-induced reduction of cytochrome b from *Dictyostelium* in vitro" (co-authored with K. L. Poff); K. Raschke on "The parasitic epidermis (no photosynthetic CO_2 reduction in guard cells)"; T. Sharkey on "The reduction of photosynthesis caused by phaseic acid" (co-authored with K. Raschke); M. Sussman on "Approaches to the synthesis of the cytokinin photoaffinity derivative, 8-azido-benzyladenine" (co-authored with H. Kende); J. Suttle on "Ethylene and flower fading in *Tradescantia*" (co-authored with H. Kende); B. Whitaker on "Respiratory oscillations associated with aggregation competence in *Dictyostelium discoideum*" (co-authored with K. L. Poff).

IX International Conference on Plant Growth Substances, Lausanne, Switzerland (August 1976): Papers were given by G. Gardner on "Binding of cytokinins to particulate fractions from protonemata of the moss *Funaria hygrometrica*" (co-authored with M. Sussman and H. Kende); H. Kende "On the role of ethylene in aging", K. Raschke on "The inhibition of proton expulsion from guard cells by abscisic acid"; J. A. D. Zeevaart on "Photoperiodic control of growth and flower formation in *Bryophyllum* as mediated by gibberellins".

D. P. Delmer presented lectures on "The origin and significance of plant glycoproteins" (co-authored with D. T. A. Lamport) and "Glycoprotein synthesis in plants: a role for lipid intermediates" (co-authored with M. C. Ericson), and chaired a session on "Cellulose degradation" at the Symposium on Cell Wall Biochemistry Related to Specificity in Host-Pathogen Interactions, Tromsø, Norway (August 1976). She also gave a paper on "The biosynthesis of cellulose and other plant cell wall polysaccharides" at the Symposium on the Structure, Biosynthesis, and Degradation of Wood at the Annual Meeting of the Phytochemical Society of North America, Vancouver, British Columbia (August 1976).

A. D. Hanson gave seminars on "Proline accumulation in relation to drought resistance in two contrasting barley cultivars" at Washington State University, Pullman, Washington and at Montana State University, Bozeman, Montana (October 1976); and on "Proline accumulation and drought-resistance in barley" at the University of Arizona, Tucson, Arizona (May 1976).

A. Haug, K. Leonards, H. Weller and L. L. Yang presented a poster paper on "Membrane structure of microorganisms" at the Symposium on the Structure and Function of Biological Membranes, Banff, Alberta, Canada (March 1976).

H. Kende presented a paper "Äthylen, Kompartimentierung und Alterung" at the Annual Meeting, German Botanical Society, Zürich, Switzerland (August 1976); and gave seminars on "Ethylene and the regulation of senescence in plants" at the University of California, Los Angeles, California (February 1976), Stanford University, Stanford, California (May 1976), University of California, Davis, California (May 1976), and University of California, Berkeley, California (May 1976).

D. T. A. Lamport presented papers "Structure, biosynthesis and significance of plant cell wall glycoproteins" at the Annual Meeting of the Phytochemical Society of North America, Vancouver, British Columbia (August 1976) and "Extensin eliminated" at the American Chemical Society Meeting, New York City, New York (April 1976).

A. Lang presented a paper "Plant regulators: survey, and effects on vegetative growth" in the Symposium "Pesticide Chemistry in the XX Century" at the American Chemical Society Meeting, New York City, New York (April 1976).

W. Lockau gave a seminar on "Photosynthetic reduction of p-(diazonium)-benzene sulfonic acid" at the Midwest Meeting on Photosynthesis, Urbana, Illinois (September 1976).

J. C. Meeks presented a paper "Glutamine formation by heterocysts of *Anabaena cylindrica*" (co-authored with J. Thomas, C. P. Wolk, and S. M. Austin) at the II International Symposium on Photosynthetic Prokaryotes, Dundee, Scotland (August 1976).

K. L. Poff gave a paper on "Thermosensory transduction by *Dictyostelium discoideum*" at the Cold Spring Harbor Workshop on *Dictyostelium*, Cold Spring Harbor, Long Island, New York (May 1976) and a seminar on "Thermotaxis in *Dictyostelium*" at the Department of Biology, University of California-San Diego, La Jolla, California (January 1976).

K. Raschke presented papers on "Effects of stress on photosynthesis mediated by plant hormones" at the Gordon Research Conference on CO₂ Fixation by Green Plants, New Hampton, New Hampshire (June 1976); "The osmotic motor of stomatal movements" and "Hunger or thirst? The plant's dilemma" at the Annual Meeting of the American Physiological Society, Philadelphia, Pennsylvania (August 1976); "Effects of plant growth substances on stomata" at a workshop on Regulation of Cell Membrane Activity in Plants, Pallanza, Italy (August 1976); and gave seminars on "How guard cells regulate the gas exchange of leaves" at the Department of Biology, Marquette University, Milwaukee, Wisconsin (May 1976); and "Are plants maximizing carbon uptake in relation to water loss?" at the Smithsonian Institution, Washington, D.C. (May 1976).

S. Rodaway presented a paper on "The mode of action of GA₃" at the Workshop and Colloquium on Protein and Nucleic Acids in Plants, Strasbourg, France (July 1976).

C. P. Wolk presented a paper on "Nitrogen metabolism and cyanobacterial development" at the Second International Symposium on Photosynthetic Prokaryotes, Dundee, Scotland (August 1976) and chaired the session on "Nitrogen metabolism". He also chaired the session on "Differentiation and development" at the E. M. B. O. Workshop on Blue-green Algae, Genetics and Physiology, Oxford, United Kingdom (September 1976), and the session on "Cyanobacteria (blue-green algae)" at the Annual Meeting of American

Society of Microbiologists, Atlantic City, New Jersey (May 1976) and gave a seminar on "Differentiation and pattern formation in filamentous cyanobacteria" at the Department of Microbiology, University of Florida, Gainesville, Florida (March 1976).

PRL SEMINAR SERIES

<u>Date</u>	<u>Speaker and Institution</u>	<u>Subject</u>
January 8, 1976	Dr. E. Cabib ¹ National Institutes of Health Bethesda, Maryland	The timing and function of chitin synthesis in yeast septum formation
January 14, 1976	Dr. W. F. Harris Oak Ridge National Laboratory Oak Ridge, Tennessee	An overview of ecological research at Oak Ridge National Laboratory
January 21, 1976	Dr. D. Nevins Iowa State University Ames, Iowa	A role for glucanases in mediating IAA-induced cell cell elongation
January 21, 1976	Dr. E. Russo Max Planck Institute for Molecular Biology Berlin, Germany	Ethylene and the avoidance response in <i>Phycomyces</i>
January 28, 1976	Dr. C. Fulton Brandeis University Waltham, Massachusetts	The multitubulin hypothesis and cell morphogenesis in <i>Naegleria</i>
February 4, 1976	Dr. P. Davies Cornell University Ithaca, NY	Investigations on the senescence of whole plants using genetic lines of peas
February 11, 1976	Dr. H. Lodish Massachusetts Institute of Technology Cambridge, Massachusetts	Messenger RNA and protein synthesis during differentiation of the slime mold <i>Dictyostelium discoideum</i>
February 18, 1976	Dr. G. Petrides Michigan State University East Lansing, Michigan	Ecological problems in the preservation of wildlife and wilderness throughout the world
February 24, 1976	Dr. B. B. Sweeney ² University of California Berkeley, California	The <i>Gonyaulax</i> clock
March 3, 1976	Dr. K. Raschke MSU/ERDA Plant Research Lab	Sweet and sour guard cells (Carbon metabolism of guard cells)
March 10, 1976	Dr. C. Foy USDA Beltsville, Maryland	Differential adaptation of plant genotypes to mineral stress (toxicity or deficiency) in problem soils
April 7, 1976	Dr. D. C. Walton State University of New York Syracuse, New York	ABA metabolism in water-stressed plants

<u>Name</u>	<u>Speaker and Institution</u>	<u>Subject</u>
April 14, 1976	Dr. R. Lunt University of California Los Angeles, California	Research at the Laboratory of Nuclear Medicine UCLA emphasizing ecological programs
April 21, 1976	Dr. F. Meins, Jr. University of Illinois Urbana, Illinois	Epigenetic changes in the cell division factor requirements of cultured tobacco cells
April 28, 1976	Dr. E. H. Everson Michigan State University East Lansing, Michigan	The role of genetics and plant physiology in wheat breeding
May 4, 1976	Dr. Gordon Browning University College Aberystwyth, Wales, U. K.	Gibberellins in chloroplasts
May 5, 1976	Dr. M. H. Zimmerman ³ Harvard Forest Petersham, Massachusetts	Long-distance transport in plants - safety vs. efficiency
May 12, 1976	Dr. C. Slayman Yale University New Haven, Connecticut	Mode of electrogenic ion transport in <i>Neurospora</i>
May 19, 1976	Dr. P. Hepler Stanford University Stanford, California	Guard cell development and function: The use of protoplasts
June 28, 1976	Dr. N. P. Voskresenskaya A. K. Timiryazev Institute of Plant Physiology Academy of Sciences of USSR Moscow, USSR	The effect of light quality on correlation between the photosynthetic and oxidative reactions in green plants
June 28, 1976	Dr. I. A. Tarchevsky Institute of Biology Academy of Sciences of USSR Kazán, USSR	The effect of temperature on photosynthesis
July 14, 1976	Dr. P. B. Nicholls University of Adelaide Adelaide, Australia	Can IAA bind to phospholipids?
July 19, 1976	Dr. A. Laisk Tartu Astrophysical Observatory Tõravere 202 444 Estonia, USSR	Kinetics of photosynthesis and photorespiration
August 1, 1976	Dr. D. J. Osborne University of Cambridge Cambridge, United Kingdom	Ethylene and cell growth

<u>Name</u>	<u>Speaker and Institution</u>	<u>Subject</u>
September 22, 1976	Dr. J. W. Anderson La Trobe University Bundoora, Victoria Australia	Enzymes of sulfate reduction in higher plants
September 29, 1976	Dr. D. D. Davies University of East Anglia Norwich, United Kingdom	The control of glycolysis
October 6, 1976	Dr. R. Dilley Purdue University West Lafayette, Indiana	Membrane conformational changes and membrane-proton interactions
October 13, 1976	Dr. J. J. Bancroft University of Western Ontario London, Ontario Canada	The biophysics, genetics and biology of a plant virus
October 20, 1976	Dr. J. Gutknecht Duke University Marine Laboratory Beaufort, North Carolina	Ion transport and turgor pressure regulation in giant algal cells
October 27, 1976	Dr. R. Quatrano Oregon State University Corvallis, Oregon	The control of the polar deposition of a sulfated polysaccharide in <i>Fucus</i> zygotes
November 9, 1976	Dr. W. Brill ⁴ University of Wisconsin Madison, Wisconsin	Use of mutants to study nitrogen fixation
November 17, 1976	Dr. S. Hendricks ² USDA Beltsville, Maryland	Aspects of dormancy in seeds
November 24, 1976	Dr. W. L. Wardell University of Maryland in Baltimore County Cantonsville, Maryland	Flower induction of vegetative plants fed snap-back deoxyri- bonucleic acid from stems of flowering plants
December 8, 1976	Dr. S. Flashman MSU/ERDA Plant Research Lab	Using selenium compounds to select mutations affecting sulfur metabolism in cultured tobacco cells
December 15, 1976	Dr. A. Marcus Cancer Research Institute Philadelphia, Pennsylvania	Protein factors and capped mRNAs in protein chain initiation

¹Joint seminar with Department of Biochemistry

²Sponsored by the Plant Research Laboratory Graduate Assistants

³Joint seminar with Department of Botany and Plant Pathology

⁴Joint seminar with Department of Microbiology and Public Health

RESEARCH REPORTS

The following reports, dealing with work much of which is still in progress, are not intended as publications, and should not be cited without specific permission by the responsible investigator.

REGULATION OF FLOWER FORMATION*

1. Promotion and Inhibition of Flower Formation in a Dayneutral Plant in Grafts with Short- and Long-day Plants

A. Lang, K. Scarbro

Experimentation on the presence of flower-inhibitory, graft-transmissible materials in photoperiodically sensitive plants was continued. Background and rationale of this work have been explained in "Plant Research '75", p. 43. A major objective was to determine whether flower-inhibiting materials exist in plants in which the existence of flower-promoting, graft-transmissible materials ('florigen' or 'flower stimulus') has been well documented. The plants used were the dayneutral tobacco (*Nicotiana tabacum*) cultivar 'Trapezond' (TRAP), the short-day tobacco cultivar 'Maryland Mammoth' (MM), and the long-day plants *Nicotiana glauca* (NS) and *Hyoscyamus niger* (an annual strain) (HYO). Flower formation in MM on long days (LD) has been induced by graft union with TRAP and other dayneutral tobaccos and with NS and HYO; flower formation in NS and HYO on short days (SD) has been obtained in graft unions with TRAP and another dayneutral tobacco, and with MM (Melchers and Lang, 1941; Zeevaart, 1958; Chailakhyan and Khazhaky, 1975; Chailakhyan et al., 1976; Lang, unpublished data). The experimental design was simple: Prior to grafting, MM (and TRAP) were maintained on LD, NS and HYO on SD; after grafting, each graft combination was subjected to either LD or SD, and the flowering response of TRAP was measured.

TRAP served as stocks throughout. The plants were defoliated and decapitated, and only one or two lateral shoots allowed to develop as 'indicator shoot(s)'. In the main experiment of the report period,

* This work represents the continuation of a joint project with Professor M. Kh. Chailakhyan, K. A. Timiryazev Institute of Plant Physiology, Academy of Sciences of USSR, Moscow. It was started during A. Lang's sabbatical leave at the institute, arranged under the scientific exchange program between the National Academy of Sciences and the Academy of Sciences of USSR, and has since been continued in East Lansing and Moscow. The work reported here was greatly aided by the MSU Department of Horticulture by generously providing greenhouse space.

the scions were grafted sideways into the young portions of the TRAP shoots, and the stocks were decapitated above the graft region, forcing an indicator shoot to grow out above the latter, but only after the graft union had been established. In this manner, inhibitory effects (apical dominance) of the scion on the growth of the indicator shoot, which may interfere with the latter's flowering response, were largely avoided, and the indicator shoot, which was kept defoliated, was fully dependent on the scion from the very beginning of its growth. The flowering response was measured as number of grafts with flowering and non-flowering indicator shoots, time to appearance of flower buds and the first open flowers, and number of nodes formed prior to the inflorescence.

The results of the main experiment are shown in Table 1; representative grafts can be seen on the cover of this Annual Report. In the control grafts (TRAP/TRAP; see cover, top left pair) the time of flower formation (flower bud appearance) was the same on both LD and SD but the number of nodes on the indicator shoots was significantly less on the former than on the latter, indicating a promotion of flower formation, although the effect was relatively small. In an earlier experiment, flower bud appearance in TRAP/TRAP grafts had also been significantly accelerated on LD as compared to SD. Thus, flower formation in these grafts may be promoted by LD, and non-grafted TRAP plants may also flower sooner on LD than on SD. However, this difference, most noticeable in suboptimal light conditions, is probably not based on a photoperiodic response but a consequence of the greater quantity of light the plants receive on LD.

When MM/TRAP grafts (see cover, top right pair of grafts) were kept on SD, i.e. with the MM partner proceeding to flower, the flowering response in TRAP was significantly accelerated (right-hand graft of this pair). When these grafts were kept on LD, with MM staying vegetative (left-hand graft of pair), flower formation in TRAP was somewhat delayed but the number of nodes on the indicator shoots was not significantly different from that in TRAP/TRAP controls on LD, i.e., there was no indication of a genuine inhibitory influence from the vegetative MM partner on flower formation in TRAP. In other, smaller grafting experiments between MM and TRAP, flower formation in TRAP on LD was delayed

Table 1. Flowering and growth responses in grafts between the dayneutral tobacco cultivar Trapezond (TRAP), the short-day cultivar Maryland Mammoth (MM) and the long-day plants *Nicotiana glauca* (NS) and *Hyoscyamus niger* (HYO). In this experiment, the scions (TRAP, MM, NS) were grafted sideways into the stem of the stock (TRAP), and the 'indicator shoot' of the latter developed from the first axillary bud above the graft union.

Combination	Photo-period	No. grafts	No. forming flowers	No. remaining vegetative	Mean No. days to first visible fl. bud	Mean No. days to first open flower	Mean length of indicator shoot (cm)	Mean No. nodes on indicator shoot
TRAP/TRAP (control)	LD	15	15	0	50	65	49	18
	SD	15	15	0	49	66	52	22
MM/TRAP	LD	16	15	1*	>48**	>51**	>46**	20
	SD	16	16	0	32	50	41	15
NS/TRAP	LD	15	15	0	23	39	45	14
	SD	15	0	15	-	-	16	36***
HYO/TRAP	LD	16	16	0	34	50	39	17
	SD	16	0	16	-	-	12	34***

* Indicator shoot growing poorly

** Indicator shoots of 3 grafts produced no visible flower buds but contained microscopic ones; growth of shoots was slow

*** Indicator shoots of 5 or 6 grafts dissected; all strictly vegetative

and the node number increased, compared with TRAP/TRAP controls on LD, but the differences, although significant (particularly that in node number) were relatively small (e.g. 62 vs. 72 days to flower buds, 24 vs. 34 nodes).

In NS/TRAP and HYO/TRAP grafts (see cover, pairs of grafts at bottom, left and right, respectively) on LD, the indicator shoots formed flower buds more rapidly and after a lesser number of nodes than in the TRAP/TRAP controls (left-hand graft of either pair). Quite in contrast, under SD (right-hand grafts) the indicator shoots remained vegetative for the duration of the experiment (90 to 94 days) and had by this time formed an average of 34 to 36 nodes while under long days they had formed 14 to 16 nodes and had by that time produced mature fruits and seeds. In addition to this effect, the growth habit of the indicator shoots of the NS/TRAP and HYO/TRAP SD grafts was altered in a very marked manner: the shoots grew more slowly, were thicker, and the internodes were considerably shorter than on LD.

In summary, when the short-day plant MM tobacco or the long-day plants NS and HYO were grafted onto dayneutral TRAP tobacco and the grafts maintained on photoperiods inductive for the respective scions, flowering in the dayneutral partner was accelerated. This result indicates transmission of flower-promoting substance(s) (florigen) from photoperiodic plants to a dayneutral plant, analogous to its transmission in the opposite direction, and thus supports the idea that the 'florigens' of short-day, long-day and dayneutral plants are either identical or at least fully interchangeable. Acceleration of flowering in dayneutral tobaccos by NS on LD has already been reported, by Zeevaart (1958) and Chailakhyan et al. (1976).

When grafts of NS or HYO on TRAP were subjected to SD, i.e. photoperiods non-inductive for the long-day scions, flower formation in the dayneutral partner was inhibited in a very marked manner while vegetative growth continued; however, the growth habit was changed, making it to some extent approach the rosette-type growth characteristic of NS, HYO, and most other long-day plants, when on short days. These results strongly indicate that the lack of flowering and the growth habit of long-day plants like NS and HYO under SD conditions are determined by

flower-inhibitory and growth-regulating material(s) which are formed in the plant under SD conditions. Thus, two types of transmissible substances involved in flower regulation exist in long-day plants--'florigen', and the flower-inhibitory and growth-regulating material(s), demonstrable under the inductive and the non-inductive photoperiodic conditions, respectively. The nature of all these substances remains unknown but the demonstration of the flower-inhibitory, growth-regulating material(s) adds a new and important aspect to the physiological regulation of flower formation at least in long-day plants.

In grafts with MM, there was no or only a relatively small inhibition of flower formation in TRAP on LD, as compared to TRAP/TRAP controls. Thus, the short-day plant MM contains no flower-inhibitory materials on non-inductive photoperiods, or if they are present either their quantity or their effectiveness, as measured in grafts with TRAP, is much below that of the flower-inhibitory substance(s) in non-induced NS and HYO.

References

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- Chailakhyan, M. Kh., Kh. K. Khazhakyan and L. B. Agimyan, Dokl. Akad. Nauk SSSR 230:1002 (1976)
- Melchers, G., and A. Lang, Biol. Zentralbl. 61:16 (1941)
- Zeevaart, J.A.D., Meded. Landbouwhogeschool Wageningen 58, No. 3 (1958)

2. Supplementary Observations

A. Lang

When grafts of NS or HYO on TRAP which had initially been maintained on SD were transferred to LD, the indicator shoots soon began to elongate more vigorously and produced flower buds in about one month, and open flower after another month. This observation indicates that the inhibitory substance(s) present in the long-day plants under SD conditions either disappear quite rapidly on LD, or that their effect is overcome by the 'florigen' formed under these daylength conditions. In another grafting experiment with whole shoots of NS and HYO, in which the scion was grafted into the cut end of the decapitated TRAP shoot and the indicator shoots (in this case below the graft union) began to grow before graft union had been consummated,

some of the indicator shoots formed flower buds. These, however, failed to develop further and, instead, one or more axillary shoots grew out and developed vegetatively and in the typical "inhibited" habit. Thus, even after flower formation had occurred the inhibitory material(s) from NS and HY0 can force the dayneutral graft partner to resume vegetative growth. When single, mature but young leaves of NS were grafted onto TRAP, flowering was inhibited and growth modified on SD, much as when whole shoots were used as scions, at least as long as the leaves remained in a healthy condition. Thus, the inhibitory "principle" is present in mature NS leaves kept on SD; this does not rule out, however, that it may also be present in younger leaves.

CELL WALL BIOSYNTHESIS AND STRUCTURE IN PLANTS; GLYCOPROTEIN

SYNTHESIS IN PLANTS

This project continues to place major emphasis on a study of the enzymic mechanisms and control of polysaccharide biosynthesis in higher plants. At present, this work centers on the biosynthesis, composition and structure of cell wall polysaccharides, with special emphasis on cellulose biosynthesis. Work also continues in a second, related area -- the mechanisms of glycoprotein biosynthesis in plants.

For our work on cell wall biosynthesis and cell wall elongation, we have chosen as our experimental system the developing cotton fiber, since it offers a number of advantages (see "Plant Research '74", p. 103)-- the most notable of which is the rapid and synchronous deposition of a cellulosic secondary cell wall. Our studies have combined both in vitro (e.g. enzyme studies) and in vivo (developmental changes occurring in vivo, pulse-labeling studies, use of inhibitors) approaches in an attempt to further our knowledge of how the cell wall of the cotton fiber elongates, paths of synthesis of the cell wall components, and of the regulation of these processes.

In the area of glycoprotein synthesis, we have continued to investigate the role of lipid intermediates in the synthesis of N-glycosidically-linked glycoproteins. For these studies we continue to use developing cotyledons of *Phaseolus vulgaris* (the kidney bean). We use this tissue because it is very active in vivo in the synthesis of vicilin, the major storage protein of this species which has been shown to be a glycoprotein containing mannose and N-acetylglucosamine N-glycosidically linked to asparagine residues in the protein.

1. Developmental and Biochemical Studies on the Cotton Fiber

(a) *Mode of Growth of the Cotton Fiber*

D. P. Delmer, C. Kulow

Somewhat surprisingly in view of the commercial importance of this cell, the mode of growth of the cotton fiber has not been determined. Does the cotton fiber grow by tip growth or by overall elongation? By analogy with other highly elongated plant cells which arise from epidermal tissue, particularly root hairs, one might expect that the cotton fiber would elongate by tip growth, yet some rather old and inconclusive results in the literature (O'Kelley, 1953) indicate that this is not the case. We have re-investigated this question using cotton fibers cultured "in vitro". Ovules, with their associated fibers, taken at mid-phase of their elongation period (8 days post-anthesis), were pulsed for 12-24 hrs with ^{14}C -glucose and chased for up to 48 hrs in unlabeled glucose. The fibers nearly doubled in length over this time interval. At varying intervals, samples were removed, placed in boiling water for 5 min, then consecutively washed in water, chloroform:methanol (2:1), methanol, and again water. The fibers were then spread onto microscope slides, heat fixed, and stained with 1% Calcafluor White, rinsed in water, and immersed in emulsion for radioautography. Figure 1 shows the results of such an experiment. Following a pulse, radioactivity is found to be incorporated nearly uniformly throughout the length of the cell wall of all fibers observed. To illustrate this, Figure 1A shows an example of an extended length of one fiber harvested after such a pulse. Cell wall analyses from our previous work (Meinert and Delmer, 1977) showed that, under the assumption that wall material was deposited uniformly throughout growth, the thickness of the cell wall remained constant throughout most of the elongation phase. Thus, if the thickness remains constant and material is deposited uniformly, the fiber must be elongating uniformly throughout its length. This conclusion is also supported by a recent report (Westafer and Brown, 1976) which indicates that Golgi, in the apparent act of secretion of wall material, are found throughout the length of the fiber during elongation. Figures 1B, C, D, and E further support this conclusion by showing that the tips of fibers remain labeled up to 48 hrs after cells are transferred to unlabeled glucose. (Analyses of isolated cell walls showed that the chase was

Fig. 1. Radioautography of Cell Wall Ghosts of Cotton Fibers. A. Long stretch of single fiber ghosts harvested after 24 hr. pulse. B. Fibers harvested after 24 hr. pulse. Note that the tip is labeled. C. Fibers after 24 hr. pulse followed by 24 hr. chase. Note that tip remains labeled. D and E. Same as C except that chase was for 48 hrs. For Figs. B-E, the picture on the right is of a field of fiber ghosts stained with Calcafluor White and viewed by fluorescence microscopy. Figures on immediate right of these are of same fields, but now viewed by dark field microscopy alone to enhance visualization of silver grains.

effective within 3 hrs in suppressing new incorporation into the wall.) Further proof that the fibers were not elongating by tip growth was obtained by spraying a fine mist of charcoal grains onto fibers and following the distribution of these grains on the fibers with time. All tips observed retained charcoal grains during growth, whereas grains behind the tip area became dispersed as the mass of fibers continued to elongate. Because of the extensive intertwining of fibers, it was not possible to follow the growth of one fiber throughout its entire length by this procedure.

References

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 Meinert, M. and D. P. Delmer, Plant Physiol. 59:1088 (1977)
 Westafer, J. M. and R. M. Brown, Cytobios 15:111 (1976)

(b) Studies on the β -1,3-Glucan of Cotton Fibers

D. P. Delmer, D. Maltby, C. Kulow

Recent work from our laboratory (Meinert and Delmer, 1977) has shown that, coincident with the onset of secondary wall cellulose deposition in the cotton fiber, there also appears a significant rise in the rate of deposition in the cell wall of a non-cellulosic 3-linked glucan. We also reported (Delmer et al., 1977; Heiniger and Delmer, 1977) the results of extensive characterization of a highly active β -1,3-glucan synthetase in cotton fibers, the enzyme presumably responsible for the in vivo deposition of the 3-linked glucan. In view of the parallel synthesis of this glucan and secondary wall cellulose, as well as reports in the literature which indicate that such non-cellulosic glucans undergo turnover in the cell wall, we are currently investigating the possibility that the 3-linked glucan serves as a precursor (either specific or non-specific) to cellulose.

Figures 2A-D show the results of further characterization of the cell wall 3-linked glucan. Cell walls were prepared from fibers harvested just after the rapid onset of secondary wall cellulose synthesis (18 days post-anthesis). Figure 2A shows a GLC separation of the permethylated derivatives of the neutral sugars from these cell walls. Peak 9 has been confirmed both by retention time and mass spectrometry to be 3-linked glucan. Figures 2B, C and D show similar profiles of the same cell wall preparation except that the walls were treated with various enzyme preparations prior to permethylation.

Fig. 2. GLC Separation of Permethylated Derivatives of Carbohydrates from Cell Walls of Fibers Harvested 18 Days Post-anthesis. A. Untreated cell walls. B. Cell walls treated for 24 hrs. with hog pancreatic α -amylase. C. Cell walls treated for 24 hrs. with *B. subtilis* α -amylase preparation which also contains a β -glucanase specific for cleaving glucans with mixed β -1,3 and β -1,4 linkages. D. Cell walls treated for 24 hrs. with *Rhizopus* endo- β -1,3-glucanase. E. Derivatives obtained from ethanol-precipitable cytoplasmic constituents.

Treatment with hog pancreatic amylase (Sigma), known to be free of β -glucanase activities (D. Nevins, personal communication), has no effect upon the level of 3-linked glucose; similarly, no effect is observed after treatment with *B. subtilis* α -amylase (Sigma) which is heavily contaminated with a β -glucanase specific for mixed β -1,3 - β -1,4 linkages. However treatment with *Rhizopus* endo- β -1,3-glucanase completely removes the 3-linked glucan from the cell wall. These results further support our earlier conclusion that the 3-linked glucan is accessible to enzymatic attack without prior removal of other wall polymers.

Earlier this year we attempted to demonstrate either by starvation or pulse-chase experiments whether this β -1,3-glucan of the cell wall was labile and thus could serve as a precursor to cellulose or other cell constituents. The results of these early experiments were however highly variable, indicating that some unknown factor(s) was perturbing the system. Recently we made a completely new observation which may explain the variability in our previous experiments. The observation is that the cytoplasm, as well as the cell wall, of these cotton fibers contains a large quantity of β -1,3-glucan. Figure 2E shows a methylation analysis of the ethanol-precipitable cytoplasmic components of cotton fibers harvested 18 days post-anthesis.

The profile shows only one major peak which has been identified as 3-linked glucose. In this particular experiment, calculations show that 3-linked glucose represents at least 10% of the dry weight of the total ethanol-precipitable components of the cytoplasm and over 80% of the total polysaccharide of this fraction. Recent results also show that the concentration of this intracellular β -1,3-glucan is strictly proportional to the in vivo rate of cellulose deposition. The intracellular concentration is quite low throughout the phase of primary wall synthesis but increases over 50-fold during the onset of secondary wall deposition; the concentration subsequently declines as the rate of cellulose deposition declines. Studies this year will be directed at furthering our understanding of the functional and/or structural role this glucan may serve in the process of secondary wall synthesis in the cotton fiber.

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(c) Other, Preliminary Studies

i) Non-degradative Fractionation of Cotton Fiber Cell Walls

A. Klein and D. P. Delmer

We have followed up on a report (Johnson et al., 1975) that cellulose can be dissolved without degradation by reacting it with formaldehyde gas in anhydrous dimethylsulfoxide. We have worked to make this reaction reproducible and we are currently investigating its potential for the non-degradative fractionation of cell wall polymers, particularly the 3-linked glucan.

ii) Intracellular Levels of UDP-glucose in Cotton Fibers

D. DeWitt and D. P. Delmer

Kinetic studies on the cotton fiber β -1,3-glucan synthetase (Delmer et al., 1977) showed that it exhibited marked activation by its substrate, UDP-glucose, indicating that variations in the endogenous level of UDP-glucose could regulate the rate of 3-linked glucan synthesis, and therefore possibly also that of cellulose synthesis. This year we have developed an assay for intracellular UDP-glucose and are beginning to measure the endogenous concentrations of this compound throughout development in the cotton fiber. Preliminary results show indeed that the level of UDP-glucose increases as the rate of non-cellulosic and cellulosic glucan deposition increases and declines to undetectable levels when glucan synthesis ceases.

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2. Glycoprotein Synthesis in Plants

(a) *Purification and Structural Characterization of the Mannolipid Intermediate in Glycoprotein Synthesis*

D. P. Delmer, C. Kulow, M. C. Ericson .

Our previous work (Ericson and Delmer, 1977) has shown that a mannolipid can be synthesized from GDP-¹⁴C-mannose using extracts from cotyledons of Phaseolus vulgaris. This mannolipid can serve as an intermediate donor for the glycosylation of protein in this material. Large-scale synthesis of this ¹⁴C-mannolipid has been achieved, and the mannolipid has been purified by chromatography in organic solvents on DEAE-cellulose and repeated deacylation and rechromatography on Sephadex LH-20. The mannolipid is eluted from DEAE-cellulose at 0.05 M in a salt gradient, consistent with the presence of a monophosphate residue. After methanolysis, the CHCl₃-methanol soluble portion of the mannolipid was analyzed by mass spectrometry. The fragmentation pattern was nearly identical to that of standard dolichol, a polyprenol containing 20 isoprenoid units. An intense ion at m/e 69 represented the ω-terminal isoprenoid residue, and repeating fragments separated by 68 mass units were observed up to m/e > 1200. MS data as well as resistance of the mannolipid to catalytic H₂ reduction and phenol treatment indicate that the α-residue is saturated. All evidence supports the conclusion that the mannolipid is dolichol monophosphate mannose. These results strongly support the conclusion that the lipid carrier shown to be involved in glycoprotein synthesis in plants has a structure quite similar to or perhaps identical with that of the carriers shown to function in glycoprotein synthesis in mammalian tissues and yeast (Waechter and Lennarz, 1976).

References

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(b) *Further Studies on the Mechanism of Glycoprotein Synthesis in Plants*

M. C. Ericson, D. P. Delmer, J. Jen

Results reported herein last year and now published (Ericson and Delmer, 1977) have demonstrated a role for a mannosyl lipid intermediate in the glycosylation of protein in cotyledons of *Phaseolus aureus*. The types of glycoprotein(s) synthesized *in vitro* are those containing sugars attached via an N-glycosidic linkage. This year we have isolated by pronase digestion the glycopeptide from the purified storage protein vicilin, the most abundant glycoprotein in our experimental tissue; the sugars present are mannose and N-acetylglucosamine (GlcNAc), and the GlcNAc is linked to asparagine via an N-glycosidic linkage. We have also isolated and characterized a lipid containing an oligosaccharide with size and composition very similar to that of the oligosaccharide attached to vicilin. Kinetic evidence indicates that this lipid-oligosaccharide is the final precursor to glycoprotein. Detailed studies on the kinetics of UDP-¹⁴G-GlcNAc incorporation into lipid-PP-GlcNAc, lipid-PP-(GlcNAc)₂, lipid-PP-oligosaccharide, and glycoprotein have been performed. In the absence of GDP-mannose, radioactivity from UDP-¹⁴G-GlcNAc accumulates in the disaccharide lipids; in the presence of GDP-mannose, radioactivity is further transferred to lipid-oligosaccharide and glycoprotein.

Further studies show that when radioactive GDP-mannose is used as the substrate, 50% of the radioactivity in the insoluble products is released by protease digestion; however, when radioactive UDP-N-acetylglucosamine is used as the substrate, 98% of the radioactivity in the insoluble products is released by protease. This indicates that although there is more than one kind of final product formed from mannose, essentially all of the insoluble product containing N-acetylglucosamine is glycoprotein in nature.

These results further clarify the pathway for the glycosylation of N-glycosidically linked proteins in plants. Further work will concentrate on attempts to demonstrate the localization and nature of the protein acceptor molecules in our *in vitro* system.

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STUDIES ON HORMONE ACTION

Work on hormone action has been pursued along two lines: studies on physiological and biochemical effects of hormones, and studies on the site of hormone action. Taking the first approach, we have continued to investigate the action of gibberellin in barley aleurone layers and the effect of ethylene in flower tissue. In both instances, biochemical experiments have been supplemented by ultrastructural studies. Research on gibberellin effects in aleurone cells is directed towards two goals. We are attempting to find out whether gibberellin-induced hydrolases are secreted in free or vesiculate form, a question which has been disputed in the literature. Further, we are investigating by quantitative methods whether gibberellin treatment leads to increased formation of endoplasmic reticulum during the lag phase, before induction of hydrolase synthesis and secretion can be observed. Research on the action of ethylene deals mainly with the question how ethylene induces its own synthesis in aging plant tissue.

With regard to the second approach, we have further probed into the interaction between cytokinins and cellular constituents. To enhance the chances of finding a cytokinin receptor, a benzyladenine photoaffinity label has been synthesized. Upon photolysis, this compound is expected to bind covalently to functional groups of the receptor molecule, thus facilitating in vivo and in vitro detection of a receptor.

1. Regulation of Senescence by Ethylene in Flower Tissue

(a) *Metabolism of Membrane Lipids in Aging Flower Tissue of Ipomoea tricolor*

P. Beutelmann, R. deZacks, H. Kende

Last year (see "Plant Research '75") we reported that the phospholipid content of aging flower tissue of the Japanese morning glory *Ipomoea tricolor* declined rapidly and that ethylene accelerated this process. These experiments have now been extended to investigations on the fate of other lipid components and the effects of silver ions and benzyladenine (Beutelmann and Kende, 1977). Cytokinins have been shown to retard senescence of flower tissue (Kende and Hanson, 1976) and silver ions are known to counteract the action of ethylene (Beyer, 1976). When phospholipids were labeled with ^{33}P , the radioactivity subsequently disappeared from the phospholipid at the same rate as the total

level of phospholipid declined. Fatty acids esterified to phospholipids showed a similar decline during senescence as did the intact phospholipids. There was no difference in the contents of steroids, glycolipids and free fatty acids between senescing and juvenile flower tissue. Benzyladenine retarded senescence of flowers and also the loss in phospholipid. This effect of the cytokinin was already visible during the night prior to flower opening and aging. This result confirms our hypothesis that processes related to senescence precede actual visible signs of aging. We also investigated the effect of silver ions on the senescence process. Aging was retarded by Ag^+ at very low concentrations. However, ethylene-induced ethylene synthesis and loss of phospholipid were not affected. This result indicates that there are ethylene-controlled processes which are not silver-sensitive or, alternatively, that inhibition of certain aging phenomena by Ag^+ occurs at a step subsequent to the initial, ethylene-mediated reaction(s).

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(b) On the Action of Inhibitors of Ethylene Biosynthesis

H. Kende, R. deZacks

The amino-ethoxy analog of rhizobitoxine proved to be a very potent inhibitor of ethylene biosynthesis in morning glory flower tissue (Kende and Hanson, 1976). We found that this compound acted as an irreversible inhibitor; this raised the possibility that it might be utilized as an affinity label. The production of the radioactive amino-ethoxy analog was achieved by feeding the tritiated, reduced derivative of the inhibitor to the appropriate strain of *Streptomyces*, which produces the analog during growth in liquid medium. The action of the amino-ethoxy analog can be counteracted to some extent by pretreating flower tissue with methionine, as originally found by Lieberman et al. (1974). It can be counteracted much more effectively by pretreatment of the tissue with homocysteine, however. Homocysteine, in contrast to methionine, is also able to induce ethylene production and to hasten aging (Hanson and Kende, 1976). Originally, it was thought that the analog competed with methionine at the enzymatic site

where methionine is converted to ethylene. Our data do not exclude this possibility since homocysteine is involved in the generation of methionine. It is conceivable that only homocysteine-derived methionine can be utilized for ethylene generation if it were localized in the same compartment of the cell as the ethylene generating system. A more direct involvement of homocysteine in ethylene generation is however not excluded.

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(c) Ultrastructural Studies on Senescing Morning Glory Flower Tissue

H. L. Phillips, H. Kende

Ultrastructural research on the morning glory flower focussed on structural changes prior to flowering, at flowering, and at the onset of senescence. In the developing flower bud of *Ipomoea* marked structural changes were observed with light microscopy and electron microscopy in a group of inner epidermal cells located on each individual rib of the corolla. Examination of sections from rib tissue fixed 2 days and 1 day before flowering, on the day of flowering, and during the course of rolling-up of the ribs showed the following major changes in these cells: 1) a two-fold increase in the size of the cells, especially in the direction perpendicular to the epidermal surface, 2) reduction in the thicknesses of the cell walls and the cuticle, 3) increase in vacuolation, from cells initially rich in cytoplasm and containing only small vacuoles to fully-vacuolated cells, 4) transition from extensive amounts of strands of parallelly stacked RER and polysome-rich cytoplasm to a peripherally-located cytoplasm containing only fragmentary isolated strands of RER and limited numbers of ribosomes, 5) incorporation of the initially-existing cytoplasm into the forming, large central vacuole by the expansion of endoplasmic reticulum, 6) reduction in the number and integrity of various cell organelles, 7) reduction in the number of Golgi body complexes and Golgi vesicles, and 8) changes in the characteristic

contents of the vacuoles. These cytoplasmic changes are thought to affect the turgor status and the ultimate integrity of the cell and thereby initially effect the opening of the flower and later its rolling-up and senescence, respectively.

(d) *Effects of Ethylene on Petals of Tradescantia hirsuticaulis*

J. Suttle, H. Kende

In continuing our investigations of the action of ethylene in senescing petals of *Tradescantia* we have pursued two lines of inquiry: (i) further characterization of the action of ethylene in petals of differing maturity, and (ii) the nature of the actual in vivo ethylene precursor. It was found that petals acquired a sensitivity to ethylene during their maturation in the bud prior to anthesis. Petals were isolated from buds one or two days prior to anthesis, and on the day of anthesis, and the effects of a high (10 ppm) concentration of ethylene were examined. Petals isolated two days pre-anthesis were insensitive to the supraoptimal concentration of ethylene. They exhibited neither a higher rate of ion leakage nor an enhanced rate of ethylene production in response to the added ethylene. Petals isolated one day prior to flower opening did show an enhanced rate of ion leakage without any significant effects on the rate of ethylene production. Petals from mature (fully open) flowers exhibited an accelerated rate of both processes and were fully sensitive to ethylene.

Time-course studies showed that in petals isolated one day prior to anthesis the rate of pigment efflux in response to the added ethylene lagged far behind the rate of ion leakage. In mature petals, the rate of efflux of both components paralleled each other through the day. Thus in the partially sensitive, immature petal the action of ethylene on membrane permeability was selective while in mature petals there was a general loss of membrane integrity in response to exogenous ethylene.

The second aspect of our research with *Tradescantia* petals dealt with the biosynthesis of ethylene. Studies further to characterize the in vivo precursor of ethylene showed that either S-methyl-methionine or methionine could be the actual precursor for ethylene synthesized on the day of senescence. These studies have also shown that the specific activity of the S-methyl-methionine pool is double that of the methionine pool on the day of fading, indicating that the overall biosynthetic

scheme in *Tradescantia* is similar to that discovered in *Ipomoea* (Hanson and Kende, 1976).

Preliminary studies utilizing two compounds (N-propyl-gallate and benzoate) which scavenge free radical molecules indicated that the biosynthesis of ethylene was dependent on the presence of free radicals as already shown by Baker et al. (1976) for tomato and avocado. Furthermore, since exogenous ethylene still elicited an enhanced rate of anthocyanin efflux in the presence of these compounds the action of ethylene did not appear to depend on the presence of free radicals.

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2. Hormone Binding in Plants

(a) *High and Low-Affinity Binding of ^3H -Benzyladenine to a Particulate Fraction from Tobacco Cells*

M. R. Sussman, H. Kende

The properties of high and low-affinity binding sites from tobacco cells that had been described earlier (see "Plant Research '75") were further characterized. The high-affinity binding site was specific for biologically active cytokinins and the dissociation constant (K_d) using benzyladenine was estimated to be 1.4×10^{-7} M with 8×10^{-9} moles of binding sites per kg fresh weight of tobacco cells (Fig. 1). The low-affinity binding site was heat stable and showed only partial specificity for active cytokinin analogs. While it could distinguish gross differences in cytokinin structure (e.g. adenine versus benzyladenine), it could not recognize differences in halogenation. Para-bromobenzyladenine, which is inactive in the growth assay and which does not bind to the high-affinity binding site, competed with benzyladenine for low-affinity binding sites. The K_d of the low affinity binding site using benzyladenine was estimated to be 7.7×10^{-6} M and the number of binding sites 4.4×10^{-7} moles per kg fresh weight of tissue (Fig. 1).

Fig. 1. Scatchard plot of p -[^3H]-BA binding at different BA concentrations to show *high-affinity* and *low-affinity* cytokinin binding sites in an 80,000 $\times g$ particulate fraction from tobacco cells and their contrasting sensitivity to a heat pre-treatment (100°C, 15 min).

(b) *Synthesis of a Cytokinin Photo-Affinity Label*

M. R. Sussman, H. Kende

In order to characterize the high-affinity cytokinin binding site further, we have explored the possibility of using a cytokinin photo-affinity label along the lines used by others successfully to tag and identify cAMP- and ATP-binding sites in various tissues (Haley and Hoffman, 1975). During the last year the potential cytokinin photo-affinity reagent, 8-azido-N⁶-benzyladenine (8-N₃-BA) was synthesized from 8-bromo-adenosine in a four-step reaction sequence with 8-azido-adenosine, 1-benzyl-8-azido-adenosine and 8-azido-N⁶-benzyladenosine as intermediates.

8-N₃-BA was found to be a fully active cytokinin in the moss protonema bioassay and slightly more active than BA in a tobacco cell division (suspension culture) bioassay. In addition, at high concentrations, where BA inhibits cell division, 8-N₃-BA did not. 8-N₃-BA was readily photolyzed by long- and short-wavelength UV light yielding product(s) which were inactive in the moss bioassay but may have had slight activity in the tobacco bioassay. These results therefore justify the future synthesis and use of radioactive 8-N₃-BA for covalent labeling of specific cytokinin binding sites.

Reference

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3. The Control of Hydrolase Synthesis in Barley Aleurone Layers

(a) *Ultrastructural Investigations of Barley Aleurone Cells*

R. D. Locy, H. L. Phillips, H. Kende

To obtain conclusive electron-microscopic data on barley aleurone layers during the lag phase prior to and during gibberellic acid (GA)-induced hydrolase secretion it is necessary to know precisely the physiological status of the layer used for microscopy. Using a flow-through chamber as described by Varner and Mense (1972), α -amylase secretion by identical half aleurone layers was monitored. At 4, 8, 12, and 24 hrs after GA addition, one of the half layers was removed from the flow-through chamber and fixed for microscopy; α -amylase secretion was continuously monitored with the other half layer. The aleurone layers chosen for

ultrastructural observations showed all very similar time courses of α -amylase synthesis and secretion. Present electron-microscopic research focusses on examining the formation of rough endoplasmic reticulum (RER) and of secretory vesicles following addition of GA. Results from these experiments are expected to clarify the role of GA in inducing formation of RER and the way by which hydrolases are secreted from barley aleurone cells.

A direct attempt is also made to establish whether α -amylase is secreted from the aleurone cells via vesicles. For this purpose, aleurone layers are homogenized gently using low-shear methods and isotonic buffers. Initially, we could confirm results of Firn (1976) that part of the α -amylase is particulate, and that it can be released from this form by detergent treatment. We also found that this particulate form of α -amylase sedimented as a distinct band in sucrose gradients. Current efforts aim at establishing the significance of the association of α -amylase with this particulate fraction.

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(b) Early Responses of Barley Aleurone Layers to Gibberellin

G. Gardner, H. Kende

One of the earliest events after treatment of barley aleurone layers with gibberellic acid (GA_3) is the enhancement of orthophosphate incorporation into phospholipid. Koehler and Varner (1973) observed that ^{32}P -labeled inorganic phosphate (^{32}Pi) incorporation into phospholipid was maximal 8 hrs after GA treatment; however, there was no indication of a specific stimulation of phosphate labeling associated with any particular subcellular fraction of the aleurone cells. Those authors suggested that the severe grinding procedures needed to insure breakage of aleurone cells disrupted organelles and thus obscured any specific effect. We hoped that isolation of purified membrane fractions would provide additional information about early events in hormone action, and we employed a double-labeling technique to help reduce variability due to fractionation procedures.

One hundred aleurone layers of Himalaya barley were treated with 10^{-6} M GA₃ for 8 hrs and then pulse-chased with $^{33}\text{PO}_4$. A second group of layers was incubated in the same fashion but without the hormone, and then pulse-chased with $^{32}\text{PO}_4$. The two groups of layers were combined and homogenized together and fractionated on discontinuous sucrose density gradients as proposed by R. L. Jones (personal communication). The $^{33}\text{P}/^{32}\text{P}$ ratio across the gradient was then determined. It was reasoned that this ratio would be constant across the gradient if the hormone effect was general or was caused by an increased ease of extraction of the GA-treated layers. However, if GA caused an enhanced phosphate incorporation into any specific fraction, one would expect an increase in the isotope ratio in that fraction. Since this measurement is relative, a decrease in ^{32}P incorporation into the control is indistinguishable from an increase in the GA-treated layers.

After 8 hrs of GA treatment, a constant and highly reproducible increase in ^{33}P incorporation relative to ^{32}P was found in a fraction which sedimented at the 30-45% (w/w) sucrose interface. Treatment of both groups of layers with GA did not yield an increased isotope ratio in this fraction; therefore, this effect was caused by the hormone and not based on an isotope artifact. This fraction was enriched for mitochondria, as observed in the electron microscope, and had maximal cytochrome *c* oxidase activity. The GA effect was not observable after only 4 hrs of treatment. The increase in isotope ratio was not observed when the fractions were extracted for phospholipid or ribonucleic acids. However, the same fraction showed an increased isotope ratio if extracted for Pi or organic P. It is reasonable to postulate that nucleotide triphosphates are the likely fraction which showed increased labeling as a result of GA treatment.

Reference

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(c) *Partial Characterization of α -Amylase Secreted by Barley Seeds*

S. J. Rodaway

α -Amylase is the major protein synthesized and secreted in response to treatment by GA. Because many secreted proteins are also glycoproteins, we analyzed the composition of α -amylase to determine if it was glycosylated. First, we had to develop a method which would allow us to test whether any sugars associated with the α -amylase protein were covalently attached to the protein. The method combines SDS-polyacrylamide gel electrophoresis and either aqueous or non-aqueous techniques of carbohydrate cleavage, followed by gas chromatographic quantification of the sugars. The technique can be used for the analysis of small amounts (<1 mg) of glycoprotein. Using this method, the α -amylase secreted by whole grains of Himalaya barley was found to contain, on the average, up to 0.5 residues per molecule of mannose, glucose and glucosamine. Because there are four isozymes of α -amylase in this tissue, this result indicates that the isozymes may be heterogenous with regard to their carbohydrate moiety. Since an estimation of the number of sugar residues per molecule of α -amylase required an accurate quantitation of the amount of α -amylase protein per sample, an amino-acid analysis of the enzyme preparation was also performed. α -Amylase contains proportionally more histidine and tyrosine and less glutamate-glutamine than the "average" protein molecule. A second protein was also found which was not synthesized during GA treatment but which had many properties similar to those of α -amylase, including partial glycosylation.

RESISTANCE OF PLANTS TO ENVIRONMENTAL STRESS

It is clear that feasible avenues to increased productivity in a wide array of farming systems include plant breeding and crop management to reduce the yield losses caused by environmental stresses (Dilley et al., 1975; Hanson and Nelsen, in press). Such approaches to plant productivity are becoming more necessary for several reasons: (a) the demand for, and use of, energy in the U.S. economy is imposing stresses directly upon farmland and forest ecosystems (e.g. by atmospheric pollution); (b) the growing competition for land and water resources between energy-producing and -consuming technologies, and agriculture causes stresses indirectly (e.g. by decreasing the availability of water for irrigation); (c) the rising costs of energy tend to promote fuel-use economies in farming operations (e.g. tillage, irrigation), which lead to increasing liability of crops to stresses; (d) the major farming regions of the U.S. may be entering a period of increased climatic fluctuations, with frequent droughts and severe winters.

There is currently a dearth of information on the metabolic changes that precede or accompany whole-plant responses to stress; this limits detection of stress effects at early stages, and is a major barrier to scientific plant breeding and crop management aimed at maximizing plant resistance to stress. It will be necessary to unravel the metabolic events - deleterious as well as protective - which occur during episodes of stress, in order to exploit the many strategies that plants have developed over evolutionary time to cope with environmental stresses.

This project has adopted two related approaches to the problems of understanding and utilizing plant responses to environmental stress: (i) fundamental research on metabolic responses of cereals to water stress, and (ii) collaborative research with the wheat-breeding program at MSU aimed at dissecting from the complex character of drought-resistance measurable physiological and biochemical components the modes of inheritance of which can be defined. Water stress was selected as the topic for both approaches because it is a very widespread and economically significant environmental stress, and because changes in plant water

status are of central importance to the damage wrought by other stresses such as freezing and salinity.

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1. Water Potential and Proline Accumulation in Relation to Drought Injury in Two Barley Cultivars

A. D. Hanson, C. E. Nelsen; E. H. Everson (MSU Department of Crop and Soil Science)

Free proline accumulates in response to water stress in attached and detached leaves of many plants under both laboratory and field conditions (Palfi and Juhasz, 1971; Singh et al., 1973; Waldren et al., 1974). It is not yet known whether proline accumulation is of adaptive value as part of a plant's strategy for withstanding drought, but Singh et al. (1972) claimed that when 10 barley varieties were subjected to the same degree of internal water stress (i.e. exhibited the same drop in leaf water potential ψ), their proline-accumulating capacity was positively correlated with their grain yield stability (Finlay and Wilkinson, 1963) under dryland conditions. Because of the practical and theoretical importance of this report, we undertook a detailed re-evaluation of the relationship between ψ , proline accumulation and drought injury (Hanson et al., 1977), using two of the 10 barley varieties originally studied by Singh et al. The two varieties selected were 'Excelsior', a drought-resistant 6-row variety, and 'Proctor', a drought-susceptible 2-row variety.

Standard methods were used for proline determination, and a recently developed instrument (dewpoint hygrometer) was calibrated for estimation of cereal leaf ψ , in collaboration with Dr. G. R. Safir of the MSU Department of Botany and Plant Pathology (Nelsen et al., 1977).

The varietal difference in drought-resistance between Excelsior and Proctor was expressed at the 3-leaf stage when plants were stressed by water-withholding or with polyethylene glycol solution, -19 bars; drought-resistant Excelsior sustained less leaf kill than drought-susceptible Proctor (Table 1).

Table 1. Leaf kill of Excelsior and Proctor barley seedlings, assessed 3 days after relieving polyethylene glycol stress which had been applied for 4 days. Results are means of data from 10 pots, each containing two Excelsior and two Proctor plants.

Variety	Leaf Kill (percent of leaf length, $\pm$ S. E.)			
	Leaf 1	Leaf 2	Leaf 3	Leaf 4
Excelsior	39 $\pm$ 1.9	30 $\pm$ 1.6	14 $\pm$ 1.5	4.1 $\pm$ 1.7
Proctor	73 $\pm$ 3.1	48 $\pm$ 2.4	32 $\pm$ 2.1	5.2 $\pm$ 2.3

During stress, a ψ gradient developed along the leaf blade, a ψ value below -30 to -40 bars indicating leaf kill. The ψ of the mid-blade zone always fell faster in Proctor than in Excelsior (Fig. 1).

Fig. 1. Representative time courses of ψ and proline content for second leaf blades of Excelsior and Proctor during polyethylene glycol stress. Data on proline contents after 7 days stress (histograms, E = Excelsior, P = Proctor) are included for comparison.

As ψ fell, free proline accumulated in leaf tissue and reached the highest concentration when leaf kill became severe; at this stage much of the proline was localized in the killed tissue. Proline always accumulated faster in Proctor than in Excelsior (Fig. 1). Upon relief of water stress, free proline levels declined in viable leaf tissue but remained very high in the drought-killed, desiccated leaf zone. We conclude: (i) that the high proline concentrations which accumulate during sudden water stress reflect near-terminal or terminal changes caused by a decline in leaf water status; (ii) that varietal differences in proline accumulation rates can be attributed to varietal differences in control of leaf water status during stress--the more desiccated the leaf, the faster the rate of proline accumulation; (iii) that proline accumulation does not seem suitable as a positive index of drought-resistance in screening methods for cereal breeding programs as had been proposed by Singh et al. (1972).

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2. Synthesis of Glycine Betaine in Water-Stressed Barley Leaves

A. D. Hanson

Glycine betaine (betaine) levels in leaves have been reported to rise in several species subjected to NaCl stress (Storey and Wyn-Jones, 1975), and a protective value for this response has been suggested (Wyn-Jones, 1972). Barley leaves drought-stressed for three days were found to contain about three times more betaine than unstressed leaves; the unstressed leaves contained approximately 200 μg betaine/g fr.wt. Stressed leaf tissue incorporated 4 to 15 times more tracer ^{14}C -formate into betaine than control tissue and in stressed tissue ^{14}C -betaine was the major soluble labelled metabolite (Table 2); stressed tissue retained more of the ^{14}C -label in soluble and insoluble products after 18 hrs incubation than did control tissue.

Table 2. Incorporation of tracer ^{14}C -formate into betaine by water-stressed and control barley leaf segments. Batches of six or eight 1 cm leaf segments were infiltrated with about 0.5 μCi of $\text{Na-}^{14}\text{C}$ formate (59 $\mu\text{Ci}/\mu\text{mole}$) per segment and incubated for 18 hrs in darkness.

Experiment No.	Pretreatment of plants	Total ^{14}C formate infiltrated dpm $\times 10^3$	Total ^{14}C activity* recovered from tissue dpm $\times 10^3$	^{14}C activity in betaine dpm $\times 10^3$
1	Well-watered	6,660	736	25
	PEG-stressed	6,660	971	321
2	Well-watered	8,880	1,587	80
	PEG-stressed	8,880	2,409	1,284
3	Well-watered	5,094	1,781	328
	PEG-stressed	6,347	2,759	1,249

* Balance of ^{14}C lost principally as $^{14}\text{CO}_2$

It is probable that betaine synthesis in plants proceeds via serine, ethanolamine and choline, and that the methyl groups of betaine can be derived from formate reduction involving THFA metabolism (Bowman and Rohringer, 1970). Two interesting points arise from the high activity of these pathways in stressed tissues: a possible inter-relationship between membrane phospholipid components and betaine synthesis, and the highly reductive nature of the pathway from formate to methyl groups - which is reminiscent of the reducing equivalent demand for proline synthesis during water-stress.

References

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SENSORY TRANSDUCTION IN PLANTS

This project is, as before, directed toward an understanding of sensory transduction in plants. In particular, we are interested in the sequence of events whereby information concerning the state of the environment is acquired by the organism through non-visual light receptions and by measurement of temperature.

Our major interest in light reception is in identifying the "blue-light" photoreceptor pigment. We have described a blue light induced cytochrome-*b* reduction and suggested that the photoreduction of the cytochrome via a flavin may be used as an assay for the photoreceptor pigment controlling the physiological blue light responses (Poff and Butler, 1974, 1975). Because of questions concerning the specificity of the cytochrome-*b* photoreduction as an assay for the physiological blue light photoreception pigment (Schmidt and Butler, 1976a,b), we have isolated the photoreducible cytochrome-*b* and investigated the relative ability of different flavoproteins to function as the photoreceptor pigment for the reduction of the purified cytochrome.

Pursuing the hypothesis that the temperature receptor controlling thermotaxis in *Dictyostelium* is the lipid component of a cellular membrane, we have initiated a study of the fatty acids in the plasma membrane of *Dictyostelium*. We have also investigated the changes in fatty acid composition of the plasma membrane when the organism is fed exogenous lipids.

References

Poff, K. L., and W. L. Butler, Nature 248:799 (1974); Plant Physiol. 55:77 (1975)

1. Cytochrome-*b* Photoreduction Catalyzed by Flavoproteins

K. Manabe, K. Poff

Last year we reported the isolation and purification of the photo-reducible *b*-type cytochrome from *Dictyostelium* ("Plant Research '75",

p. 111). Recently we have studied the efficiency of several diverse flavoproteins in serving as the photoreceptor for the photoreduction of this purified *b*-cytochrome.

The procedure used for purification of the *b*-cytochrome has been previously given. A flavoprotein preparation was partially purified from *Dictyostelium* through chromatography on Sephadex G-25, Sephadex G-100 and DEAE cellulose following concentration with Amicon pressure dialysis of a high speed (144,000 x g for 60 min) supernatant fraction from a broken cell brei. Photoreduction of the *b*-cytochrome was monitored spectrophotometrically as described by Poff and Butler (1974) in a reaction mixture containing a constant amount of purified cytochrome-*b*. The results (Table 1) show a great deal of variability in the capacity of various flavins for participating in the photoreduction of the purified cytochrome-*b*. These data thus support the view that the photoreduction of cytochrome-*b* is a relatively specific photo-reaction in vivo.

Table 1. Relative ability of various flavoproteins to catalyze the photoreduction of cytochrome-*b* purified from *Dictyostelium*.

Flavoprotein	Concentration of test flavoprotein ¹ (μM)	<i>b</i> -Cytochrome photo-reduced (% of total)
Partially purified flavoprotein from <i>Dictyostelium</i>	0.6	73
	0.125	27
D-Amino acid oxidase	4	74
	2.5	64
L-Amino acid oxidase	2.5	23
	0.5	5
Diaphorase	0.5	7
Control	0 ²	0

¹The complete reaction mixture contained 1 mM potassium phosphate buffer pH 7.0, 0.4 μM cytochrome *b*, 5 mM EDTA, 1 μM glucose oxidase, 0.25 mg catalase, 2 mg D-glucose.

²It should be noted that the reaction mixture contains glucose oxidase, catalase and glucose to reduce the oxygen concentration. Thus, the control contains a flavoprotein (glucose oxidase) which however does not catalyze the photoreduction of the cytochrome *b*.

Reference

Poff, K. L., and W. L. Butler, Nature 248:799 (1974)

2. Modification of the Fatty Acid Composition of Plasma Membranes of *Dictyostelium discoideum*

B. Whitaker, K. Poff

Cells of *D. discoideum*, Ax-2, were grown on a medium containing protease peptone, 5g; trypticase, 5g; yeast extract, 5g; Na_2HPO_4 , 0.35g; KH_2PO_4 , 0.35g in 1000 ml of medium, or in the same medium using lipid free yeast extract (extracted for 24 hrs with chloroform methanol, 2:1, and 24 hrs with diethyl ether) with 10 mg/l stearic acid, 2 mg/l folic acid and 200 $\mu\text{g/l}$ lipoic acid. Plasma membranes from cells harvested at the exponential phase of growth were broken with a Dounce-type tissue homogenizer and were purified by aqueous phase separation (Burnett and Till, 1968) and sucrose density gradient centrifugation.

A chloroform-methanol (2:1) extract of the purified membrane fraction was washed, dried under nitrogen and methyl esters were prepared by base catalyzed esterification (Luddy et al., 1968). Samples were separated by gas chromatography on a Supelcopore SP 2300 80/100 column. The major modification in the lipid composition of the purified membranes caused by growth on stearic acid is on the relative amounts of oleic (18:1), linoleic (18:2), and linolenic (18:3) acids. The stearate feeding results in an 80% decrease in lanoleic acid and an 85% decrease in oleic acid compared to the linolenic acid content of the membrane. The decrease in oleic and linoleic acids is balanced by an increase in linolenic acid.

References

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Cuddy, E. F., R. A. Barford, S. F. Herb, and P. Magidman,
J. AOCS 45:549 (1968)

STOMATAL REGULATION OF THE GAS EXCHANGE BETWEEN PLANT AND ENVIRONMENT

The stomatal apparatus is the plant's prime and specific control element for the regulation of the exchange of water vapor and CO_2 with the environment. Stomatal functioning affects the efficiency by which plants use sunlight, atmospheric CO_2 , water and, indirectly, nutrients found in the soil or supplied as fertilizer. Knowledge of stomatal functioning is necessary for an estimation of the minimal water requirement for plant production and for an assessment of the potential of plants as energy converters for man.

The stomatal control system responds to environmental factors through the action of several feedback loops. The responses are mediated by physiological factors within the plant (the intercellular CO_2 concentration; turgor; concentration of abscisic acid (ABA), a plant hormone formed rapidly under stress).

Stomata adjust the fluxes of CO_2 and water vapor through osmotic swelling and shrinking of the guard cells which surround the stomatal pores. The most important osmotica involved are the chloride and the α -malate of potassium.

During the past year the following aspects of stomatal functioning were studied: i) the change in the magnitude of the feedback of the intercellular CO_2 concentration brought about by the stress hormone abscisic acid; this effect may play a role in the optimization of carbon uptake in relation to water loss (item 1, p. 56); ii) the presumed movement of ABA as feedback messenger from the mesophyll to the epidermis (test of a method to be employed; item 2, p. 58) and the possible metabolism of ABA to an inhibitor of photosynthesis (item 3, p. 59); iii) the roles of transfer of inorganic and metabolism of organic anions in the operation of the stomatal turgor mechanism (items 4, 5, pp. 60, 61); iv) the possible role of malic acid as a mediator of the stomatal response to CO_2 (item 6, p. 62). Item 7, p. 63 , reports on attempts to employ electrophysiological methods in the study of transducer and effector mechanisms operating in guard cells.

1. Further Measurement of CO₂ Loop Gains and of Effects of ABA on these Gains

D. Dubbe, G. D. Farquhar, K. Raschke

We reported on the effects of abscisic acid (ABA) on the loop gain of the feedback loop involving CO₂ and stomata ("Plant Research '75", p. 32). This loop consisted of a physiological gain (dP/dC_i) and an environmental gain $(\partial C_i / \partial P)_{c,A}$ (where C_i = intercellular [CO₂], P = stomatal permeability, and c = ambient [CO₂]). In the case of *Xanthium strumarium* and cotton (*Gossypium hirsutum*), increased levels of ABA greatly increased the absolute values of the physiological, environmental, and loop gain.

We have continued this study by examining more species, including the C₄ dicotyledon, *Amaranthus powelli*. We also separated the feedback loop involving CO₂ and stomata into two subloops, one involving CO₂ and stomata, and the other involving CO₂ and net assimilation (A). This allowed us to examine the response of intercellular [CO₂] to changes in the stomatal permeability which is independent of changes in net assimilation. Previously, the feedback loop involving CO₂ and net assimilation was incorporated into the environmental gain. Thus, if we wish to examine how feedback involving stomata, assimilation, and CO₂ would affect a change in intercellular [CO₂] resulting from a small change in ambient [CO₂], the appropriate equation would be:

$$\Delta C = \frac{\Delta C \text{ in the absence of feedback}}{1 - \text{loop gain of the permeability loop} - \text{loop gain of the assimilation loop}}$$

Each loop gain is composed of a physiological gain (dP/dC_i) and (dA/dC_i), respectively, and a physical gain $(\partial C_i / \partial P)_{c,A}$ and $(\partial C_i / \partial A)_{c,P}$.

We have determined the gains for five species of which we have experimental data (Table 1). In all cases, increased ABA levels resulted in increases of the loop gain of the assimilation loop. In all cases except *Zea mays*, increased ABA levels also increased the loop gain of the permeability loop. This is a quantitative statement of the earlier finding of a sensitization of stomata to CO₂ by ABA. In the three C₃ plants investigated and in *Amaranthus powelli* the CO₂ control loops are relatively unimportant when the plants are unstressed. Under conditions of stress, the importance of these loops increases dramatically. The stomata of *Zea mays* appear to be

Table I. The gains of the feedback loops involving permeability, assimilation, and CO₂ in the absence and presence of 10⁻⁵M (±)-ABA in the transpiration stream.

Species	ABA?	Physiological Gains		Physical Gains		Loop Gains	
		$\frac{dP}{dc_i}$	$\frac{dA}{dc_i}$	$\frac{\partial ci}{\partial P c, A}$	$\frac{ci}{A c, P}$	Permeability Loop	Assimilation Loop
		10 ² mol m ⁻² s ⁻¹	10 ⁻² mol m ⁻² s ⁻¹	10 ⁻⁵ m ² s mol ⁻¹	m ² s mol ⁻¹	(dimensionless)	
<i>Xanthium strumarium</i>	No	0.00	3.75	1.99	-2.21	0.00	-0.17
	Yes	-34.44	5.00	33.1	-6.53	1.14	-0.33
<i>Gossypium hirsutum</i>	No	-2.50	2.20	8.45	-3.67	-0.02	-0.08
	Yes	-4.17	3.64	74.6	-11.85	-0.31	-0.43
<i>Avena sativa</i>	No	0.00	3.33	7.72	-2.83	0.00	-0.09
	Yes	--	No steady values attained		-	-	-
<i>Zea mays</i>	No	-33.30	1.84	107.89	-11.40	-3.59	-0.21
	Yes	-5.88	3.33	146.67	-12.10	-0.86	-0.40
<i>Amaranthus powelli</i>	No	-8.00	0.00	57.62	-8.42	-0.46	0.00
	Yes	-11.00	1.33	182.48	-14.50	-2.01	-0.19

sensitive to CO₂ at all times.

2. Extraction of ABA from *Commelina communis* Epidermis

M. Pierce

Stomata close in response to water stress; ABA levels rise in leaves in response to water stress; exogenous ABA closes stomata. One can infer from these facts that increased ABA in water-stressed leaves closes their stomata. Nevertheless a physical association between ABA and epidermal tissue has never been established.

Abaxial epidermis was stripped from turgid and wilted leaves of *Commelina communis* for ABA extraction. Each epidermis was examined by microscope, and adhering mesophyll cells were cut away. The ABA extraction method according to Zeevaart (1974) was scaled down to permit determination of ABA in the batches of epidermis, which were less than 20 mg in dry weight.

The mesophyll tissue plus adaxial epidermis was extracted too. ABA levels in the water-stressed leaves were 3 to 8 times higher than in the control leaves (0.70 ng ABA/mg dry weight compared to 0.15 ng ABA/mg dry weight). The smallness of the putative ABA peaks in the gas chromatograms (GC) for the epidermal samples permitted only semi-quantitative evaluation of their ABA contents. There was no clear difference in ABA content between the epidermal samples from turgid and wilted leaves. The level was roughly 0.3 ng ABA/mg dry weight.

That the GC peaks truly represent ABA was demonstrated in two ways. The samples remaining after GC injection were isomerized by two days of exposure to intense fluorescent light to an approximately 50:50 mixture of ABA and the 2-*trans*-ABA isomers. Reinjection of the samples on the gas chromatograph showed reduced peaks at the proper retention time for ABA and the appearance of new peaks at the proper retention time for 2-*trans*-ABA.

The other method involved the development of a bioassay for ABA based on closure of stomata in *Commelina communis* epidermal strips. Isomerized ABA samples were diluted to estimated concentrations of 0.5×10^{-8} M or 0.5×10^{-9} M in (+)-ABA based on the GC data. The ability of these solutions to cause stomatal closure was compared with that of authentic ABA solution of known concentrations. All solutions contained 10 mM MES buffer, pH 4.2. The biological activities of the extracts agreed with their estimated ABA contents; it is possible to demonstrate

the presence of ABA in epidermis.

Reference

Zeevaart, J.A.D., Plant Physiol. 53:644 (1974)

3. Inhibition of Photosynthesis by Phaseic Acid?

T. D. Sharkey, K. Raschke

Kriedemann et al. (1975) reported that the metabolite of abscisic acid, phaseic acid (PA), reduced photosynthesis in leaf sections and whole leaves. We have previously found ("Plant Research '74", p. 26) that stomata of three species were insensitive to a plant extract containing PA while those of *Commelina communis* closed. This year, we tested the fraction of a plant extract believed to contain PA (as indicated by thin layer and gas chromatography) on its action on photosynthesis in leaf slices and isolated mesophyll cells. A 5×10^{-5} M solution of the presumed PA inhibited photosynthetic O_2 evolution completely or almost completely in all of the 8 species tested (*Arachis hypogaea*, *Vicia faba*, *Brassica oleracea*, *Spinacia oleracea*, *Amaranthus powelli*, *Xanthium strumarium*, *Commelina communis*). ABA had no effect on photosynthesis.

To determine whether the effect of presumed PA is on the photosynthetic electron transport chain, the rate of the Mehler reaction was measured in isolated mesophyll cells of *X. strumarium* in the absence and presence of PA. There was no inhibition of this reaction (using methyl viologen or benzoquinone as electron acceptors) which means that the extract does not interfere with electron transport up to the high energy acceptor of photosystem I.

Further purification of the plant extract containing PA yielded two fractions, one inhibiting photosynthesis, the other not. The presumed PA is in the inactive fraction. The isolation and unequivocal identification of PA as well as of the inhibitor in the active fraction is in progress.

Reference

Kriedemann, P. E., B. R. Loveys and W.J.S. Downton, Austr. J. Plant Physiol. 2:553 (1975)

4. Chloride Affects Malate Accumulation during Stomatal Opening in Epidermal Strips of *Vicia faba*

C. A. Van Kirk, K. Raschke

K^+ is known to accumulate in guard cells to increase turgor pressure when stomates open. However, there has been less certainty as to the anion accompanying K^+ into guard cell vacuoles. Cl^- has been observed to accumulate but the extent of its participation has varied from 5% (in *Vicia faba*) to 100% in individual stomatal complexes of *Zea mays*. In addition, it is known that anions need not be supplied to guard cells during opening: epidermal strips floating on solutions of K^+ salts of impermeant acids open as widely as they do on solutions of KCl. This fact, combined with the observation that the starch content of guard cells often decreases during opening, suggested that organic anions might be serving to balance K^+ during stomatal opening.

In 1973, Allaway showed that epidermal malate could balance about 1/2 of the K^+ accumulated. We tried to relate this observation to reports of Cl^- participation in the stomatal mechanism (e.g. Raschke and Fellows, 1971) by investigating the effect of chloride on malate accumulation in epidermal strips.

Epidermal strips were made from leaf sections of *Vicia faba* having nearly closed stomates. The strips were then floated on solutions of ~100 meq K^+ and varying proportions of Cl^- and iminodiacetate (IDA^-). (The latter is an impermeant zwitterion). The strips were placed in the light and humidified air containing either zero CO_2 or "ambient" CO_2 (assumed to be about 300 $\mu l\ l^{-1}$) was passed over the strips. After 4 hours, the strips were removed from these solutions; stomatal aperture was measured under the microscope and the tissue was extracted in boiling 80% ethanol. The extracts were then assayed for malate according to a procedure modified from the method of Goldberg and Passonneau (1974). In epidermal strips exposed to Cl^- -free solutions the relationship between malate content and stomatal aperture was nearly linear. When Cl^- was available, the linear relationship was no longer observed. We speculate that Cl^- suppresses malate formation by circumventing a rise in the intracellular pH associated with the uptake of K^+ in the absence of a permeant anion.

References

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Methods of enzymatic analysis. Vol. 3. Academic Press,
New York (1974)
- Raschke, K., and M. P. Fellows, Planta 101:296 (1971)

5. The Fate of Malate during ABA-Induced Closure

C. A. Van Kirk, K. Raschke

When stomates close, guard cells must dispose of the osmotica they have accumulated in their vacuoles during opening. K^+ and whatever Cl^- has been utilized move into subsidiary cells if these are present. Inorganic ions may also be held by the cell wall or specialized epidermal cells which seem to store ions. Results from ^{14}C labelling studies (Dittrich and Raschke, 1977) indicated that organic anions may be disposed of in 3 ways: (1) catabolism in the tricarboxylic acid cycle; (2) decarboxylation followed by gluconeogenesis and starch formation; and (3) release from the epidermis. Studies with unlabelled malate cannot distinguish between the first two processes but can demonstrate release from the epidermis. To this end two types of experiments on the fate of malate during ABA-induced closure were undertaken.

In the first type of experiment, ABA at three concentrations was supplied to epidermal strips of *Commelina communis* taken from leaf sections with open stomates. After 30 or 40 minutes exposure to various concentrations of ABA in light and CO_2 -free air, stomatal aperture was measured, and the tissue extracted and assayed for malate. The solution on which the strips had floated was also collected, evaporated, and analyzed for malate.

With increasing concentrations of ABA, stomates closed more rapidly. The malate remaining in the epidermis declined, and increasing amounts of malate were released to the bathing medium. In *C. communis*, epidermal cells remain intact after stripping; thus, malate released represents a net loss from the intact epidermis. The sum of malate remaining in the tissue and malate released to the solution increased with increasing degrees of closure. Strips floating on water also closed somewhat

during the experiment. In this case, too, the malate content of the epidermis decreased from the initial level and malate appeared in the bathing solution.

In the second type of experiment, epidermal strips of *V. faba*, whose stomates were initially open, were floated on solutions of 10^{-4} M ($\pm$)-ABA for periods of 15 minutes to 2 hrs. After these times, apertures were measured and the tissue and bathing solution were analyzed for malate. The malate content of the epidermis decreased in parallel with stomatal aperture.

The results of these experiments support the conclusion reached on the basis of labelling studies. Release of malate from guard cells during stomatal closure is a significant process allowing a rapid reduction in stomatal aperture during the stomatal regulation of gas exchange. ABA accelerates the discharge of malate but does not produce an effect qualitatively different from stomatal closure occurring in the absence of ABA.

Reference

Dittrich, P. and K. Raschke, Planta 134:77 (1977)

6. Stomatal Closure Initiated by Short-Chain Organic Acids, in Particular Malic Acid

K. Raschke, C. Schmuck

Results reported in "Plant Research '73", (p. 25 et seq.) led to the formulation of a hypothesis according to which malic acid has a dual role: (i) In the vacuole, it serves as counter ion for K^+ ; (ii) In the cytoplasm, it determines the direction of ion transport. If present at high concentrations, the pH is lowered. It is postulated that the combined effect of low pH and high malate leads to ion loss from the vacuoles and the guard cells: stomata close (Raschke, 1975).

We attempted to introduce organic acids into guard cells from outside. Epidermal strips with open stomata were taken from leaves of *Commelina communis* and *Vicia faba* and floated on solutions of various organic acids at a pH corresponding to their (first) pK's (in order to provide the acids partly in the protonated form which is probably the form in which a small acid penetrates the plasmalemma). The strong acid, HCl, as well as the presumably non-permeating zwitterionic 2-(N-morpholino)ethanesulfonic acid (MES) applied at the same pH as the organic acids tested, served

as controls. Both plant species tested responded to short-chain acids (acetic, glutamic, glycolic, malic, succinic) by stomatal closure; stomata did not close on comparable solutions of MES and HCl. The species differed in stomatal sensitivity. For instance, stomata of *Commelina communis* began closing 15 minutes after exposure to 100 mM malate and were completely closed after 45 minutes. Stomata of *Vicia faba* required a concentration of 300 mM malate for an onset of the response after 15 minutes.

In further experiments the malate concentrations were held constant and the pH was varied. Malate initiated closure only if given at pH's around or below the first pK (3.4), indicating that only the completely protonated acid entered the guard cells.

The results obtained are consistent with the proposed hypothesis.

Reference

Raschke, K., Planta 125:243 (1975)

7. Experiments on the Electrophysiology of Stomatal and Epidermal Cells of *Commelina communis* and *Vicia faba*

R. Saftner, K. Raschke

Recent research on the osmotics of stomatal movement indicates that changes in the osmotic pressure of guard cells which are produced by an influx of potassium (K^+) and chloride (Cl^-) ions and an accumulation of organic anions are responsible for stomatal opening. Measurements of the electrical potential across the stomatal complex during the movement and accumulation of ions is necessary to identify the passive and energy-dependent processes occurring during stomatal regulation of gas exchange.

We made ultrafine microelectrodes (tip diameter, 0.2 μm or less) and measured the electrical potential differences between both epidermal and stomatal cells, of epidermal strips and the bathing solution. An electrical potential difference exists between guard cells and surrounding cells both when the stomata are open and when they are closed. This electrical potential difference is maintained in a variety of bathing solutions. Results obtained with *Commelina communis* differ from those of Penny and Bowling (1974). These authors claimed that no appreciable electrical potential difference existed between the cells of the stomatal complex and the surrounding epidermal cells. However, they used large-tipped microelectrodes (tip diameter about 1 μm) which upon penetration of guard cells lead to a loss of turgor, an indication of cell damage. Furthermore,

we have observed that when a guard cell gradually loses turgor (as evidenced by cell collapse) after a microelectrode penetration, the electrical potential difference across the stomatal complex also drops to zero.

Upon stomatal opening, the electrical potentials of stomatal cells become hyperpolarized relative to the bathing solution. In addition, smaller electrical potential difference may develop between the subsidiary and epidermal cells (in *Commelina communis*). Lastly, the electrical potentials of cells of closed stomata are independent from the method used to close the stomata, whether stomata shut in response to passing 10^{-5} M ($\pm$)-ABA, CO_2 -containing solutions, or solutions of low ionic strength over the epidermal strips, or in response to a dark treatment.

Table I. Electric potentials of epidermal and stomatal cells in epidermal strips of *Vicia faba* and *Commelina communis* (relative to 5 mM K^+ salts of zwitterionic buffers; no correction for tip potentials)

Species	Cell Type	Potential (mV)	
		Open Stomata	Closed Stomata
<i>V. faba</i>	Epidermal	-23	-30
	Guard	-72	-30
<i>C. communis</i>	Epidermal	-46	-15
	Subsidiary	-49	-20
	Guard	-73	-41

Reference

Penny, M. G., and D.J.F. Bowling, Planta 119:17 (1974)

DEVELOPMENT AND NITROGEN FIXATION IN FILAMENTOUS CYANOBACTERIA

Filamentous cyanobacteria (blue-green algae) form multicellular patterns by mechanisms termed "fields of inhibition" and "inductions" in developmental biology. Thus, differentiated cells called heterocysts inhibit nearby vegetative cells from becoming heterocysts (Wolk, 1967) and, in *Anabaena cylindrica*, induce adjacent vegetative cells to sporulate (Wolk, 1966). Because these organisms have three types of cells (vegetative cells, heterocysts, and spores or akinetes), they permit comparison of alternative differentiation processes. Studies of pattern formation in these morphologically simple organisms may serve as models for investigations of pattern formation in higher organisms, both plants and animals.

Aerobic fixation of dinitrogen (N_2) is known to take place to a substantial extent, and perhaps only, in the heterocysts, and is dependent on a cooperation between these cells and the vegetative cells. The latter supply heterocysts with products of photosynthesis and receive, in return, products of N_2 fixation.

The goals of this research project are (i) to elucidate the biochemical bases of pattern formation, and (ii) to pursue applications of photosynthetic nitrogen fixation by these organisms. We are therefore developing use of the isotope ^{13}N as a tool for analyzing the intercellular interactions underlying pattern formation and photosynthetic nitrogen fixation (1, below); are developing the genetics of *Anabaena* as a tool for modifying development, for manipulating the N_2 -assimilating capacity of the organism, and for analyzing the mechanisms underlying nitrogen fixation (2, below); and are, with lesser emphasis, pursuing other routes which may help us to reach the stated goals (3, below).

References

Wolk, C. P., Am. J. Bot. 53:260 (1966); PNAS 56:1246 (1967)

1. Metabolic Studies Using ^{13}N

C. P. Wolk, J. Thomas, J. C. Meeks, P. W. Shaffer; S. Austin, A. Galonsky,
W.-S. Chien (MSU Cyclotron Laboratory)

(a) Metabolism of $^{13}\text{NH}_4^+$

In order to elucidate certain of the interactions whereby heterocysts and vegetative cells cooperate in the fixation of atmospheric nitrogen, and which may possibly be involved also in the intercellular control of pattern formation, we have developed techniques for studying nitrogen metabolism using $[^{13}\text{N}]\text{N}_2$ (Wolk et al., 1976) and $^{13}\text{NH}_3$. ^{13}N generated as described previously, was converted to $^{13}\text{NH}_3$ (ca. 5 mCi) by a Kjeldahl procedure modified from the method of Carangal and Varner (1959).

The principal initial product of metabolism of $^{13}\text{NH}_4^+$ by N_2 -grown or NH_4^+ -grown *Anabaena cylindrica* was found to be amide-labeled glutamine. The specific activity of glutamine synthetase is approximately half as great in NH_4^+ -grown as in N_2 -grown *A. cylindrica*. In the presence of methionine sulfoximine, an inhibitor of glutamine synthetase, formation of glutamine is essentially completely inhibited; direct formation of glutamate takes place, but at only a few percent of the normal rate of formation of that amino acid, and alanine is formed about as rapidly as glutamate. Azaserine, an inhibitor of amide transferases, reduces formation of $[^{13}\text{N}]\text{glutamate}$ approximately 100-fold, without significant effect on the formation of $[^{13}\text{N}]\text{glutamine}$. During a pulse-chase experiment, ^{13}N is transferred from the amide group of glutamine into glutamate, and apparently into the α -amino group of glutamine. On the basis of these results and others in the literature, it was concluded that the glutamine synthetase/glutamate synthase pathway mediates most of the initial metabolism of ammonium in *A. cylindrica*, and that glutamic acid dehydrogenase and alanine dehydrogenase have, quantitatively, only a very minor role.

References

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Wolk, C. P., J. Thomas, P. W. Shaffer, S. M. Austin and A. Galonsky,
J. Biol. Chem. 251:5027 (1976)

(b) *Nitrogenous Products of Heterocysts*

Isolated heterocysts accounted for up to 34 percent of the acetylene reducing activity of whole filaments, and had a specific activity of up to 1560 nmoles C_2H_4 formed $\cdot$ (mg heterocyst chlorophyll) $^{-1} \cdot \text{min}^{-1}$. The formation of $[^{13}N]NH_3$ and $[^{13}N]$ glutamine from $[^{13}N]N_2$ by such heterocysts is inhibitable by acetylene. Isolated heterocysts also formed ^{13}N -labeled glutamine from $[^{13}N]NH_4^+$ and glutamate, and ^{14}C -labeled glutamine from NH_3 and $[^{14}C]$ glutamate, in the presence of MgATP. Methionine sulfoximine strongly inhibited these syntheses.

Glutamate synthase is, after nitrogenase and glutamine synthetase, and third sequential enzyme involved in the assimilation of N_2 by intact filaments. However, the kinetics of solubilization of the activity of glutamate synthase during cavitation of suspensions of *A. cylindrica* indicate that very little, if any, of the activity of that enzyme is located in heterocysts. Concordantly, isolated heterocysts failed to form substantial amounts of radioactive glutamate from either $[^{13}N]$ glutamine or $[^{14}C]\alpha$ -ketoglutarate in the presence of other substrates and cofactors of the glutamate synthase reaction. However, they formed $[^{14}C]$ glutamate rapidly from $[^{14}C]\alpha$ -ketoglutarate by aminotransferase reactions, with various amino acids as the nitrogen donor.

It therefore appears that glutamine newly synthesized in heterocysts is transported to vegetative cells, where glutamate is synthesized. It further appears that part of the glutamate formed then returns to the heterocysts to be amidated or, alternatively, that α -ketoglutarate and aminated substrates of the aminotransferase of heterocysts react within heterocysts to form glutamate which is then amidated.

It is possible that the substance that is produced by heterocysts and that inhibits vegetative cells from differentiating into heterocysts may be glutamine or a derivative or glutamine.

2. Mutants

T. Currier, J. Haury, C. P. Wolk, P. W. Shaffer, W. Lockau

(a) *Isolation*

Auxotrophic photosynthesis-defective mutants of *Anabaena variabilis* were isolated by a method in which, after mutagenesis and before penicillin enrichment, mutant and wild type cells were separated by cavitation.

Auxotrophs were identified by their inability to grow on minimal medium and were partially characterized by replica plating to media supplemented with single nutrients or specific groups of nutrients. Of the 83 auxotrophs isolated, 65 required an inorganic source of nitrogen for growth. In addition, auxotrophs were isolated which required methionine (six), uracil (two), adenine (one), biotin (two) and nicotinic acid (two). (The number of isolates of each type is indicated in parentheses.) The nutrient requirements of five auxotrophs appeared complex and were not determined. Non-photoautotrophic mutants, of which six were isolated, were identified by their ability to grow in the dark with fructose, but inability to grow in the light without sugar.

A large proportion of the mutants requiring inorganic fixed nitrogen was altered in the differentiation of heterocysts. The following morphological aberrancies were observed: abnormally high and abnormally low frequencies of heterocysts; thick, uneven heterocyst envelopes; incompletely developed pore regions; very distinct pore regions; and protoplasts separated from the envelope of the heterocyst.

Spontaneously occurring, N_2 -fixing, prototrophic revertants of mutants with aberrant heterocysts have been isolated at a frequency of 2×10^{-8} to 4×10^{-8} of the cells plated. The fact that most such revertants produced morphologically normal heterocysts is consistent with the idea that heterocysts play an essential role in aerobic N_2 fixation.

(b) *Preliminary Characterization*

(i) Characterization of mutants incapable of growing with N_2 as nitrogen source provides a means of identifying the processes essential for N_2 fixation in cyanobacteria. The flow-sheet shown in Table 1 has been used for preliminary characterization of such mutants. To date, mutants defective in assimilation and defective in protection against O_2 have been tentatively identified. (ii) Mutants requiring methionine or uracil have been tested for growth in the presence of metabolic precursors of their nutritional requirements, thereby determining which enzymes leading to those required substances are functional in vivo in the mutants. Earlier enzymes in the pathways are then assayed in vitro. By means of this approach, the corresponding metabolic lesions have been narrowed down to a small number of possibilities, but no lesion has yet been definitively identified.

Table 1. Flow-sheet for preliminary characterization of nitrogen fixation (NF) mutants.

1. Do whole filaments of the mutants reduce acetylene aerobically at a large fraction of the wild-type rate?
Yes: Presumably reduces N_2 to NH_3 , but cannot assimilate NH_3 when at low concentration.
No: Go to 2.
2. Do whole filaments of the mutants reduce acetylene microaerobically at a large fraction of the wild-type rate?
Yes: Defective in O_2 -protective mechanism.
No: Go to 3.
3. Do whole filaments of the mutants reduce C_2H_2 anaerobically with dithionite at a large fraction of the wild-type rate?
Yes: Defective generation of reductant (or possibly very defective in O_2 -protective mechanism).
No: Go to 4.
4. Do broken filaments of the mutants reduce acetylene, in the presence of dithionite, MgATP, and light, at a large fraction of the wild-type rate, per heterocyst?
Yes: Defective generation of ATP.
No: Defective or deficient in one or both proteins of the nitrogenase enzyme complex.

(iii) Mutants capable of at most very slight growth in the light without sugar but which grow well in the dark with sugar were tested in vivo for photosystem II activity by the reaction $\text{H}_2\text{O} \xrightarrow{h\nu} \text{p-benzo-quinone}$, for photosystem I activity by the reaction $\text{diaminobenzidine} \xrightarrow{h\nu} \text{methyl viologen}$, and for combined photosystem II + photosystem I activity by the reaction $\text{H}_2\text{O} \xrightarrow{h\nu} \text{methyl viologen}$. Light-stimulated fixation of $^{14}\text{CO}_2$ was also measured, as were in-vivo absorption spectra.

Mutants PA 1, 2 and 3 had very low activity of photosystem (PS) II and reduced activity of combined photosystems II + I, and showed reduced content of biliproteins and reduced $^{14}\text{CO}_2$ fixation, but normal PS-I activity, relative to wild type *A. variabilis*. The lesion in these mutants appears to involve PS II. Three other mutants (PA 4, 5 and 6) show essentially normal rates of in-vivo reactions for PS II, PS I and PS II + I, and a normal in-vivo absorption spectrum. These mutants are presumed to be defective in some dark reaction.

3. Other Approaches

J. C. Meeks, T. Hirose, W. Lockau, P. W. Shaffer, C. P. Wolk;
R. Malmberg (MSU Department of Crop and Soil Sciences)

(a) *Uptake of Cyanobacterial Cells by Protoplasts of Higher Plants*

With the eventual aim of developing new strains of higher plants capable of N_2 fixation, we have begun the attempt to establish a stable endosymbiosis between auxotrophic mutants of N_2 -fixing *Anabaena variabilis* and cultured cells of *Nicotiana*. In the initial phase of this work, we have determined conditions under which cyanobacterial cells are taken into plant cell protoplasts, and have observed that the "endosymbiosis" soon deteriorates.

The methodology we use for uptake is based in part on that employed by Bennett and Eriksson (1974) for uptake of chloroplasts, by Giles and Whitehead (1976) for uptake of *Azotobacter*, and by Kao et al. (1974) for intergeneric fusion of protoplasts. Cyanobacterial filaments, 50 to 100 cells in length, are reduced to 1- to 2-cell fragments by mild sonic treatment, and are suspended in protoplast culture medium containing 0.3 M mannitol. Protoplasts are derived from either dark-grown cell suspension cultures or from leaves of aseptically-grown albino mutant plants of *Nicotiana* by means of enzymatic digestion of the cell wall. Protoplasts, ca. 10^6 per ml, are suspended with ca. 10^8 cyanobacterial

fragments per ml for 15 to 20 minutes in ca. 30% (w/w) polyethylene glycol (PEG) 4,000. During this time, cyanobacterial cells are taken up by 5 to 6% of the protoplasts. The PEG is then removed. Plant cell walls regenerate in 3 to 5 days in liquid culture, and cyanobacterial cells outside of plant cells are then lysed by treatment with lysozyme. The plant cells are then resuspended in liquid culture or plated on culture medium solidified with agar.

A decline in the number of protoplasts containing cyanobacterial cells occurs for 5 to 7 days following uptake until essentially none are observed. We are currently attempting to increase the frequency of uptake of cyanobacteria, and to determine the reason(s) for the decrease in number of endosymbionts.

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(b) Studies on Sporulation of Cylindrospermum licheniforme

Sporulating cultures of *C. licheniforme* secrete a substance which stimulates sporulation of new inocula (Fisher and Wolk, 1976). More than 90% of the activity in the original supernatant can be extracted from lyophilized supernatant fluid with absolute methanol. The activity cannot be extracted with a more polar solvent such as ether. The methanolic extract was fractionated by paper chromatography in isopropanol-formic acid-water (20:1.1:5, v/v). After chromatography, five ninhydrin-positive bands and three UV-fluorescent bands can be detected. Sporulation-stimulatory activity is recovered in the region of $R_f \geq 0.8$, in which are present two UV-fluorescent bands.

Substances common among extracellular products of algae were tested for their ability to stimulate sporulation. Aspartate, proline, phenylalanine, and isoleucine are active, and 1 mM tryptophan is very stimulatory. None of these amino acids could be detected within the active region of the chromatograms.

Reference

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(c) *Electron Transfer to Nitrogenase in Isolated Heterocysts of Anabaena cylindrica*

It is possible to isolate apparently intact heterocysts from *A. cylindrica* with an active nitrogenase in the complete absence of dithionite (Thomas et al., 1977). Heterocysts isolated by this method can be used to search for physiological electron donors to nitrogenase in these cells, by assaying for reduction of acetylene in the presence of ATP and such donors. NADPH, in the absence of any regenerating system, was found to be unable to support acetylene reduction by such preparations of heterocysts. However, if the substrate of the oxidative pentose phosphate cycle, glucose-6-phosphate, is added to the cell suspensions, in the presence of catalytic amounts of either NADP^+ or NADPH, but not in their absence, acetylene reduction is observed. These results confirm observations made by Peterson and Burris (1976) with a different strain of *Anabaena*. The observed rates of the glucose-6-phosphate-dependent reduction of acetylene were typically 15%, and maximally 40%, of the dithionite-supported rates, and varied considerably from preparation to preparation. Preliminary results indicate that the rate-limiting step in this system is not the NADPH (re)generation. The glucose-6-phosphate-dependent acetylene reduction occurs in the dark.

In the absence of any added NADP(H), diaminodurol, an artificial electron donor for photosystem I (PS I), supports a strictly light-dependent reduction of acetylene. The rates of the diaminodurol-dependent photo-reduction and the glucose-6-phosphate-dependent (dark) reduction of acetylene are approximately equal. We tentatively attribute this observation to the occurrence of an intermediate, presumably ferredoxin, common and rate-limiting to both pathways of electron transfer to acetylene. Our attempts to find a physiological substance capable of serving as an electron donor to PS I in heterocysts have thus far been unsuccessful. The scheme shown below indicates possible, proposed pathways of electron transfer to nitrogenase in isolated heterocysts of *A. cylindrica*.

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(d) Heterotrophic Macrocultures of Anabaena

A. variabilis grows heterotrophically in fermentors, under nitrogen-fixing conditions, with a doubling time of ca. 36 hrs (Fig. 1) at 30°C, or as low as 25 hrs at 34°C. If 5 mM fructose is originally present, the culture achieves a density of close to 4 μ g chlorophyll/ml (0.4 mg dry weight/ml), consistent with about a 60% efficiency of conversion of fructose carbon to cell carbon. If additional fructose is added subsequently, densities of greater than 10 mg dry weight/ml are attained. Growth is as rapid, but not as extensive, in the presence of sucrose. The densities obtained in the dark with fructose are greatly in excess of the densities achieved in photoautotrophic fermentor cultures, which depart from exponential growth at about 2.5 μ g chlorophyll/ml. Because heterotrophic growth is not subject to light limitation, *Anabaena variabilis* can potentially be grown to high density in very large volumes. The duration of the initial period of growth at low densities can be reduced by supplying light; the macroculture may then be darkened, and fructose added.

Fig. 1. Growth curve of a 12-1 fermentor culture of *Anabaena variabilis* in the dark at 30°C, in the medium of Allen and Arnon supplemented initially with 5 mM fructose, and inoculated from a 50-ml heterotrophically grown, flask culture. Additional supply of fructose, at 20 days (arrow) and thereafter, permits exponential growth to over 100 μg of chlorophyll/ml.

ENVIRONMENTAL CONTROL OF PLANT GROWTH AND DEVELOPMENT THROUGH HORMONES

Environmental factors such as daylength, temperature and water deficit have a pronounced effect on plant growth and development. Hormones often function as intermediaries between perception of an environmental factor and the morphological manifestation. Thus, a complete understanding of environmental effects on plants must include knowledge of the nature of the hormones involved, and how their rates of biosynthesis and metabolism are controlled by the environment. The following three problems are being studied in whole plants: (a) Flower formation which is induced by the floral stimulus. The aim is to determine the nature of this stimulus. In addition the role of translocation is inhibitory effects on flowering is under investigation. (b) Stem and leaf growth as regulated by gibberellins (GAs); here we are mainly concerned with the nature of the hormones, and their distribution in the various organs and cell fractions. (c) Wilting which is the result of a water deficit and associated with increases in abscisic acid (ABA) synthesis and metabolism. The aim is to determine the sites of ABA synthesis and metabolism in plants under normal and under stress conditions.

1. Analysis of the Estrogen-like Fraction in Higher Plants

L.L.L. Van Rompuy, J.A.D. Zeevaart

Estrogens have been reported to be present in several plant sources (Dean et al., 1971), and the appearance of estrogen-like compounds has been associated with the formation of flower buds (Kopcewicz 1971, 1972). However, estrogen or estrogen-like compounds were never identified by unambiguous criteria. We have analyzed flowering plant material of *Phaseolus*, *Perilla* and *Chenopodium* by mass fragmentography and failed to detect any estrogens, i.e. steroids containing a phenolic A ring and saturated B, C and D rings. Instead, as isolated by the Kopcewicz procedure, the estrogen-like fraction contained monoglycerides of fatty acids ("Plant Research '75", p. 47). We now report on the analysis of the estrogen fraction from apple seeds and date pollen which reportedly contain estrone (Bennett et al., 1966; Gawienowski and Gibbs, 1969). The materials were analyzed according to the method of Bennett et al. (1966) which involves

extraction of the acidic fraction and isolation of the ketonic fraction with the Girard T reagent. The efficiency of the isolation procedure for estrone was tested by adding ^{14}C -estrone to the Soxhlet extract prior to partitioning between CH_2Cl_2 and 2 N NaOH. Contrary to expectations most of the radioactivity was recovered in the neutral fraction instead of in the acidic fraction. The radioactivity recovered co-chromatographed with authentic estrone on thin-layer plates in two solvent systems. When ^{14}C -estrone was partitioned between 2 N NaOH and CH_2Cl_2 in the absence of plant material, 79% of the label was recovered in the CH_2Cl_2 phase (neutral fraction). After the Girard T extraction, the neutral fraction was purified by thin-layer chromatography and analyzed by combined gas chromatography-mass spectrometry. No estrone could be detected. Instead, methyl esters of fatty acids, monoglyceride-acetonides, and β -sitosterol were identified. The latter compound had, in the TLC-systems used, Rf-values identical to those of estrone.

The ketonic extracts from apple seeds were also analyzed for estrone by radio-immunoassay. This method indicated a level of less than 2 ng/250g seeds. However, since interfering substances can affect the readings in this assay, these results cannot be taken as conclusive evidence for the presence of estrone. The presence of estrone in plants remains therefore to be demonstrated by rigorous chemical criteria. Although cholesterol is widespread in the plant kingdom, it is apparently not metabolized to C_{18} steroid hormones as it is in animals. In plants cholesterol can be converted to poly-hydroxylated sterols such as ecdysones (Heftmann, 1975).

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2. Translocation in *Xanthium* in Relation to Long-Day Inhibition of Flowering

C. Cetas, J.A.D. Zeevaart

The objective of this study was to investigate the possible role of translocation in long-day (LD) inhibition of flowering in the short-day plant *Xanthium*. Induced leaves or parts thereof were labeled with $^{14}\text{CO}_2$ and the movement of ^{14}C -labeled assimilates out of the leaves to the shoot tips was determined by autoradiography and by counting the amount of radioactivity that accumulated in the shoot tips. In parallel experiments the flowering response was measured in plants that received the same photo-periodic treatments as the labeled plants.

(a) *Induction of a Single Leaf with the Other Leaves Remaining in Long Days*

Induction of the half-mature leaf 3 (counting from the shoot tip in basipetal direction) in the presence of other leaves in LD did not inhibit the flowering response of the terminal shoot apex. Gibby and Salisbury (1971) reported that leaf 2 kept in LD completely suppressed the flower-inducing effect of leaf 3, but we have been unable to confirm this observation. Thus, when the floral stimulus moved upward, no inhibitory effect of LD leaves could be detected under our conditions. The fact that flower formation can be induced by a single leaf in the presence of several non-induced leaves strongly argues against the idea that flowering depends on a particular ratio of several substances (e.g. Vince-Prue, 1975). It would seem unlikely that such a ratio could be established and maintained in the shoot apex by a single induced leaf in the presence of several other non-induced leaves that would "export" an unfavorable ratio.

When the floral stimulus moved in downward direction from leaf 3 to an axillary shoot at node 7, mature leaves located between the induced leaf and the receptor bud had a marked inhibitory effect on flowering and at the same time reduced the amount of ^{14}C -assimilates that accumulated in the bud when leaf 3 was fed with $^{14}\text{CO}_2$ (Table 1). These results demonstrate that assimilates along with the floral stimulus originating in the induced leaf did not reach the receptor shoots in sufficient quantities because LD leaves provided an alternate supply of assimilates without stimulus.

Table 1. Inhibitory effect of LD leaves positioned between the induced leaf and the receptor bud at node 7 on flowering response and accumulation of ^{14}C -assimilates in *Xanthium*. Leaf 3 labeled with $^{14}\text{CO}_2$. Axillary shoots harvested after 6 hrs.

Leaves in LD	Floral stage	Radioactivity in axillary shoot, dpm/mg dry wt.
None	5.5	160,950
Leaf 5	2.7	26,810
Leaves 5 and 6	0.8	23,640

(b) *Induction of Various parts of a Single Leaf*

It has been reported earlier ("Plant Research '74", p. 45) that induction given to the basal half of a leaf caused normal flowering whereas the effect of the induced apical half was strongly inhibited by the LD basal half. However, induction of a lateral half, with the other half remaining in LD, resulted in a normal flowering response. Thus, in order to be inhibitory, LD tissue must be located between the induced part and the receptor bud.

In $^{14}\text{CO}_2$ feeding experiments, more ^{14}C was translocated to the shoot tip from the basal than from the apical leaf half. Autoradiographic studies indicated that ^{14}C -assimilates were channeled out of the apical leaf half through the major veins and midrib without migrating to other portions of the blade. There was never any indication in autoradiograms that ^{14}C -assimilates moved from the basal to the apical leaf half.

Various treatments that could reduce or eliminate the LD inhibition were given to the LD basal half. In parallel experiments $^{14}\text{CO}_2$ was fed to the apical leaf half to see if translocation to the shoot tip was correlated with the flowering response. Removal or darkening of the LD basal leaf half enhanced export of ^{14}C -assimilates and also promoted flowering (Table 2).

Table 2. Influence of various treatments given to the LD basal leaf half on flowering and on accumulation of ^{14}C -assimilates in shoot tips of *Xanthium* after labeling of the apical leaf half.

Treatments started at end of the 16 hr dark period given to the apical leaf half. Harvested 4 hr after labeling. Since the experiments were performed at different times, data can be compared only between paired treatments.

Treatment of LD basal leaf half	Floral stage	Radioactivity in shoot tip, dpm/mg dry wt.
Intact	0	147,740
Removed ^a	4.7	216,830
Light	0.1	86,080
Darkness	4.7	157,510
Intact	0.8	36,470
Cut between apical and basal half	0.7	24,610
Intact	0.3	50,570
Cut along midrib	4.0	227,740

^aBasal half removed except midrib and major veins.

Cutting the mesophyll between the apical and basal half reduced the flowering response, and at the same time lowered the amount of radioactivity that accumulated in the shoot tip. On the other hand, cutting the LD basal half along the midrib had a strong flower-promoting effect, and increased the amount of radioactivity in the shoot tip.

It is clear from these results that any treatment that decreased or eliminated the flower-inhibiting effect of the LD basal half at the same time increased the amount of ^{14}C -assimilates that was translocated from the induced apical half to the shoot tip. However, as was also the case with whole plants (Table 1), there was no strict quantitative relationship between the flowering response and the amount of radioactivity that accumulated in the shoot tip. Preventing translocation from the basal leaf half was obviously crucial in eliminating LD inhibition (Table 2). For example, cutting the LD basal leaf half along the midrib allowed translocation out of the apical half while blocking it from the non-induced half. It appears

therefore that the LD base inhibits flowering because the LD leaf area supplied assimilates to the shoot apex that dilute the floral stimulus. However, the involvement of a mobile inhibitor that counteracts the effect of the floral stimulus in the shoot apex cannot be completely ruled out.

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3. Compartmentation of Gibberellins in Spinach in Relation to Photoperiod

J. Metzger, J.A.D. Zeevaart

Spinach is a long-day rosette plant which responds upon transfer from short-day (SD) to long-day (LD) conditions with increased petiole growth and stem elongation. It has been established in earlier work (Zeevaart, 1971) that these responses are mediated by gibberellins (GAs). However, the levels of GAs extracted from whole plants did not correlate with the observed physiological responses (Zeevaart, 1971). Therefore, control of stem and petiole growth is not a simple function of presence or absence of GAs. This led us to propose that it is the distribution, i.e. "compartmentation", of GAs within the plant which is the important factor that must be considered, rather than the total GA content. A change in photoperiod would cause a redistribution of GAs and this in turn would be responsible for the physiological responses.

This hypothesis is being investigated by analyzing different plant "compartments" for their GA content as a function of photoperiod. There are at least two levels at which one can approach this problem: firstly, on a gross organ level, and secondly on the subcellular or organelle level.

(a) Compartmentation on an Organ Level

Spinach plants (cv. Savoy Hybrid 612) were grown hydroponically in half-strength Hoagland solution supplemented with extra nitrogen (1.5g KNO₃/l) in SD. When the plants were of sufficient size, a portion of them was transferred to LD. At the end of the treatment roots and leaves were harvested separately and their GA levels determined by standard methods (Zeevaart, 1971). The GA content as affected by different photoperiodic treatments is shown in Figure 1 for the shoots, and in Figure 2 for the roots. In agreement with

Fig. 1. Effect of daylength on the levels of endogenous gibberellins in spinach shoots. Thin-layer chromatography of acidic fractions on Silica-gel H developed in chloroform-ethyl acetate-acetic acid (60:40:5; v/v). Ten zones of each chromatogram tested with d-5 corn bioassay.

Fig. 2. Levels of gibberellins in roots of spinach plants subjected to short or long days. Compare Fig. 1 for gibberellins in shoots.

previous work with shoots the level of a yet to be completely characterized dihydroxy-GA (an isomer of GA₁) at Rf 0.1-0.3 decreased with increasing number of LD. During the same period the level of GA₂₀ (Rf 0.4-0.6) increased. Roots, on the other hand, showed no such drop in the dihydroxy-GA level with increasing number of LD; in fact there may be a slight increase in the amount of this particular GA under LD as compared to SD. More importantly, however, is the complete lack of GA₂₀ in root extracts. The GA detected in roots could have been synthesized in situ, or it could have been imported from the leaves. We are investigating this question further by analyzing excised root tips grown in culture, and by studying GA transport between the shoot and root system.

(b) Compartmentation on a Subcellular Level

To study GA levels on a subcellular or organelle level, one must not only be able to isolate the structures in sufficient quantity and purity, but also with adequate rapidity so that leakage of small molecules such as GAs is held to a minimum. Because these criteria present technical problems, we first isolated and analyzed chloroplasts, since these can be isolated in sufficiently large quantities with relative ease.

Spinach leaves were ground in a Waring blender with a buffered isotonic sucrose solution at pH 8 (Railton and Reid, 1974). The brei was filtered through cheesecloth and then subjected to differential centrifugation, yielding a chloroplast pellet. The pellet was frozen, lyophilized and extracted in methanol. The extracts were purified and assayed for GAs as usual. As shown in Figure 3, the chloroplast fraction was enriched in GA₂₀ as compared to the whole leaf extract. When expressed per unit weight of chlorophyll, 10-15% of GA₂₀ in the leaves was associated with the chloroplast fraction, but only 1% of the dihydroxy-GA. This could mean that GA₂₀ is synthesized in the chloroplasts, and interconverted to other GAs at some other site in the cell. There is evidence with other systems that GAs are synthesized in etioplasts (Cooke et al., 1975) and in chloroplasts (Stoddart, 1968). Further work is directed towards localizing some of the pivotal enzymes in GA biosynthesis, as well as towards isolating other cell compartments for GA analysis.

Fig. 3. Comparison of endogenous gibberellins in spinach leaves under long days and in the chloroplast fraction prepared from comparable leaves.

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4. Absciscic Acid Synthesis and Metabolism in *Ricinus* and *Xanthium*

C. Cetas, J.A.D. Zeevaart

(a) *Analysis by High-performance Liquid Chromatography (HPLC)*

Analysis of absciscic acid (ABA) and its metabolites phaseic acid (PA) and dihydrophaseic acid (DPA) in previous work (Raschke and Zeevaart, 1976; Zeevaart, 1977) involved partitioning into ethyl acetate at pH 2.5, purification and separation by preparative thin-layer chromatography (TLC), and quantitative determination by gas-liquid chromatography (GLC) with electron capture (EC) detector. We have now developed a more rapid method using HPLC for the purification procedure.

Samples of leaf material (up to 10g fresh weight) were extracted with methanol. The combined extracts were dried, taken up in 10 ml of 0.2M phosphate buffer at pH 8.2 and the lipids removed with petroleum ether. The aqueous residue was lyophilized and the residue suspended in 4.0 ml of 20% ethanol in 0.2 N acetic acid. Following filtration through a Millipore filter, the clear extract was injected into a 5-ml loop connected to a series of 4 columns (each 60 cm x 0.65 cm) packed with Bondapak C₁₈/Porasil B. The sample was eluted with a linear gradient of 20-50% ethanol in 0.2 N acetic acid in 30 minutes and a flow rate of 9.9 ml/min. With this reverse phase system DPA was eluted first, and ABA last. After lyophilization the ABA samples were further purified with a micro Bondapak-NH₂ column (30 x 0.4 cm) using a linear gradient (40-100%) of ethyl acetate containing 1% acetic acid in hexane in 10 minutes and a flow rate of 2 ml/min. The PA and DPA samples were run through the same column but the gradient started at 80% ethyl acetate. Following methylation the level of each compound was determined by GLC with EC detector. Recovery of ¹⁴C-ABA added to samples was approximately 70%.

(b) *Metabolism of Abscissic Acid in Ricinus and Xanthium*

We have previously studied the sites of ABA biosynthesis and metabolism in *Ricinus* by analysis of mature leaves (sources), shoot tips (sinks) and phloem exudate in turgid and stressed plants. On the basis of the data obtained we arrived at the conclusion that ABA is predominantly produced in mature leaves, and that ABA itself as well as its metabolites are translocated in the phloem to the shoot tips where DPA accumulates as the end product of the metabolic pathway. Thus, ABA metabolism can take place both in young and in mature leaves of *Ricinus* (Zeevaart, 1977). This conclusion has been further substantiated by feeding ^{14}C -ABA via the petioles to detached leaves. In both young and mature leaves the bulk of the labeled ABA was metabolized to ^{14}C -PA and ^{14}C -DPA over a 24-hr period.

In *Xanthium* the ABA content of leaves decreases with age (Raschke and Zeevaart, 1976). It is not clear, however, whether this is because of a high rate of ABA synthesis in situ, or to import from older leaves. Since phloem exudate cannot be collected directly from cuts in *Xanthium* stems, leaves were cut from stressed plants and the cut surface treated with EDTA for 1 hr. Exudate was then collected in distilled water (King & Zeevaart, 1973). Both ABA and PA were present in the exudate in a concentration of approximately $5 \times 10^{-6}\text{M}$, but no DPA was detected. This latter metabolite is present in leaves in very low levels compared to ABA and PA. When ^{14}C -ABA was fed to detached *Xanthium* leaves, most radioactivity was present in PA, and very little in DPA.

Further studies on synthesis and metabolism of ABA in *Xanthium* were performed with detached mature leaves that were stressed by reducing their fresh weight by 10% under a stream of hot air from a fan. The level of ABA in these leaves increased rapidly during the first 4 hrs and then remained constant; it decreased after 24 hrs. If such leaves were supplied with water after 8 hrs, the ABA level dropped rapidly to that of turgid leaves. These results indicate that there may be two mechanisms for ABA synthesis: (a) a mechanism in normal, turgid leaves, and (b) a mechanism that becomes operative only in stressed leaves.

Transient increases in the levels of PA in wilted leaves have also been observed, but DPA was under all conditions present only in trace amounts. This indicates that in *Xanthium* DPA is either further metabolized rapidly, or there is an alternate route for breakdown of PA.

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BIOCHEMISTRY OF SULFUR DIOXIDE INJURY IN PLANTS

Plants are more sensitive to SO_2 than are people, and crop production decreases in proportion of SO_2 injury. Thus the impact of increases in atmospheric SO_2 on crop productivity, especially near large centers of coal combustion, may be a very serious environmental consequence of the projected increase in the nation's dependence on coal during the next few decades. It is therefore important to develop the fullest possible understanding of a) how SO_2 injures plants, and b) the devices available to plants for protecting themselves against injury by SO_2 .

We are studying the comparative physiology and biochemistry of genetically and phylogenetically related plants which differ in sensitivity to SO_2 . We believe that this approach is likely to generate new insights into mechanisms of injury by SO_2 and resistance to SO_2 . Our efforts are concentrated on the Cucurbitaceae because there is a relatively wide range of variation in SO_2 sensitivity in this family.

1. Assays of Injury by Sulfur Dioxide

R. A. Bressan, L. LeCureux, P. Filner

The traditional method for assaying injury is to estimate visually the percent of leaf area which is necrotic on a large number of leaves, and then calculate the mean of the estimates. Although this method works quite satisfactorily provided that the estimator is consistent and that a large number of leaves are used, it has the disadvantages of being subjective, of being unsuitable for resolving small differences in injury, and of being inconvenient when the effects of large numbers of treatments on injury are being determined and compared. Consequently, we have been seeking an alternative method of assay.

Leaves exposed to bisulfite at injurious concentrations emitted ethylene, and so we explored the possibility of using ethylene emission as the injury assay. It was judged unsatisfactory, however, for the following reasons. First, control leaves also emitted ethylene, although at lower rates. Second, release of ethylene reached a maximum as a function of bisulfite concentration before injury had become maximal, and then declined as injury increased further, so that at highly injurious concentrations of bisulfite, little ethylene was emitted.

Third, the time of onset of increased ethylene emission, the rate of increase of the emission rate and the time of maximum ethylene emission all depended on bisulfite concentration, so that the only feasible way of using ethylene emission as an assay appears to be to measure total ethylene emitted, rather than the rate of emission or the amount of emission up to a particular time. Measurement of total ethylene emitted requires measuring the amount of ethylene as a function of time for several times, to be sure that emission had ceased. Although ethylene emission can be used as an assay, it seems to be affected by too many variables to be used in a simple, straightforward way.

In the course of the work on ethylene emission, another gaseous emission from leaf discs exposed to bisulfite was observed, and eventually identified as ethane. In contrast to the ethylene emission, which declines at highly injurious doses of bisulfite, the ethane emission increases monotonically with increasing injury. Furthermore, uninjured tissue emits practically no ethane. The possibility of using ethane emission as an assay for injury is being explored further..

Leaves exposed to bisulfite lose their green color, so we also considered chlorophyll loss as a potential assay of injury. Initially, this method appeared to be unsatisfactory because the chlorophyll loss was rather variable. By using punched leaf discs, and then randomizing them in the treatments, variability could be reduced greatly. The sensitivity of the assay can be maximized by using only putatively sensitive leaves and by performing the incubations in light, which also bleaches the brown background color left after chlorophyll loss. In this assay, leaf discs, 8 mm in diameter, are incubated in test solution, 1 ml/disc for 2 hrs, then postincubated under fluorescent light for 24 hrs, drained, boiled in 80% ethanol, 0.5 ml/disc, and reextracted with 80% ethanol. Extracts are pooled, brought to a final volume of 1 ml/disc, and A_{655} is determined. The fraction of chlorophyll lost is computed from a standard curve made from varying numbers of discs incubated in water only. When this assay is done with sensitive leaves, 90% or more of the chlorophyll is lost as a result of a 2-hr incubation with 5 mM sodium bisulfite.

Experiments are in progress to determine how the response of discs taken from leaves fumigated with SO_2 compares to that of discs floated on bisulfite solutions.

2. Apparatus for Studying Absorption and Emission of Volatile Sulfur Compounds by Plants

R. A. Bressan, L. LeCureux, L. G. Wilson, P. Filner

An apparatus has been constructed around a Monitor Labs Model 8450 Sulfur Analyzer. The apparatus begins with a gas purification and mixing system, which is used to remove oil and volatile sulfur from compressed air, after which the desired concentration of SO_2 is mixed into the purified air. This gas mixture is passed through a plexiglas fumigation chamber, which may be a large one suitable for holding several plants, or a small one suitable for holding a single leaf, either attached to a plant or detached. The exiting air is passed through a water trap to remove SO_2 and is then returned to the atmosphere. Sampling lines are connected immediately before and immediately after the fumigation chamber. The sampling lines to be monitored are selected by timer-actuated solenoid valves, which make it possible to monitor up to six lines sequentially for times as short as a minute or as long as an hour. The gas from the sampling line is diluted with sulfur-free air to keep the volatile sulfur concentration within the linear range of the instrument and then is allowed to enter the sulfur analyzer. The analyzer responds linearly between 0 ppb and 1000 ppb SO_2 in air, with a sensitivity of 2 ppb. The data are recorded on a strip chart 25 cm wide moving at 2.5 cm/hr.

By passing sulfur-free air through the plant or leaf chamber, it is possible to use the same apparatus (except that dilution prior to analysis is not needed) to monitor emission of volatile sulfur from plants or leaves. By means of suitable traps, it is possible to discriminate between, e.g., H_2S and SO_2 .

In its original form, the apparatus included no control or measuring devices for CO_2 , water vapor or temperature. Present plans include the installation of a CO_2 trap in the air line, followed by a manifold for adding controlled concentrations of CO_2 , and a CO_2 analyzer to monitor CO_2 entering and leaving the fumigation chamber simultaneously with SO_2 measurements.

Electronic sensors are also to be installed to monitor humidity differences and temperature differences before and after the fumigation chamber. These additional capabilities will enable us to determine if the observed differences in SO_2 absorption between cultivars of Cucurbitaceae are paralleled by differences in stomatal behavior, as indicated by CO_2 absorption or H_2O emission. We also expect to be able to determine if SO_2 absorption is limited by stomatal aperture, since we should be able to alter the stomatal aperture by varying the CO_2 concentration. In preliminary experiments, we have observed that SO_2 absorption obeys Fick's First Law of Diffusion up to a certain concentration, above which the rate of SO_2 absorption is rather constant. We are particularly interested to determine if the limitation of SO_2 absorption rate is a reflection of stomatal behavior; if it differs between resistant and sensitive cultivars; and if it changes during leaf development.

3. Differences among the Cucurbitaceae in Resistance to Sulfur Dioxide

R. A. Bressan, L. LeCureux, L. G. Wilson, P. Filner

(a) *Differences attributable to Inherited Differences in Absorption of Sulfur Dioxide*

National Pickling cucumber is a cultivar of *Cucumis sativus* L. which carries germ plasm common to cultivars used in the northern U.S. SC 25 is an inbred line of cucumber which carries germ plasm common to cultivars used in the southern U.S. Commercial F_1 hybrids are often constructed by crossing northern by southern germ lines. Dr. Larry Baker, MSU Department of Horticulture, had observed that SC 25 and other lines of southern origin grow poorly in greenhouses, and he suspected that they might be more susceptible to pollutants than many northern cultivars and lines. We measured the dependence of injury on SO_2 concentration to which the cultivars were exposed, and found that to obtain 50% necrosis of the leaf surfaces, National Pickling must receive about 2.5 times the exposure to SO_2 as SC 25. In a similar comparison of two cultivars of *Cucurbita pepo* L. (Small Sugar pumpkin and Prolific Straightneck squash), a sensitivity difference was also observed. The pumpkin was the most resistant of the four cultivars, and required about 8 times as intense an exposure to SO_2 .

as cucumber SC 25 to develop necroses covering 50% of the leaf area.

The differences in sensitivity of the four cultivars correlated with differences in rate of absorption of SO_2 by the whole plant. In fact, when percent necrosis was plotted as a function of SO_2 absorbed, rather than SO_2 exposure, the data from the four cultivars became virtually indistinguishable. It therefore appears that the major factor in genetically determined differences in sensitivity to SO_2 among the Cucurbitaceae is the rate of SO_2 absorption under the exposure conditions used in the comparisons. A cultivar which is more sensitive to SO_2 absorbs SO_2 more rapidly than a less sensitive cultivar.

(b) Resistance to Sulfur Dioxide which Depends upon the Developmental Stage of the Leaf

Early investigators of SO_2 effects on plants observed repeatedly that young leaves sustained less injury than mature leaves. We have observed this phenomenon in all of the Cucurbitaceae which we have studied. A casual examination of the pattern brings to mind two hypothetical "reasons" for it: 1) an increase in sensitivity with physical distance of the leaf from the apex, or 2) an increase in sensitivity with age of the leaf. By comparing the gradient of sensitivity in slowly growing plants ($0.13 \text{ leaf day}^{-1}$) with that in rapidly growing plants ($0.27 \text{ leaf day}^{-1}$), it became clear that sensitivity increases with leaf age, not with distance from the apex. The midpoint in the sensitivity gradient was leaf number 4 in the slowly growing plant, but leaf number 8 in the rapidly growing plant. These leaves were estimated to be 31 and 29 days old, respectively.

The resistant, young leaves sustained far less injury than the sensitive, mature leaves even when leaves which had absorbed equivalent amounts of SO_2 per unit area were compared. Therefore, the resistance of young leaves is not attributable to a lower rate of absorption.

Young, unexpanded leaves have far more cells per unit area than do expanded leaves. This raised the possibility that the SO_2 absorbed per cell, rather than per unit area, determined injury, and young leaves were resistant because the SO_2 absorbed was distributed over more cells per unit area. This explanation was ruled out because fully expanded young leaves are far more resistant than fully expanded mature leaves.

4. Light-Dependent Emission of Hydrogen Sulfide from Plants

L. G. Wilson, R. A. Bressan, P. Filner

De Cormis (1) discovered that plants exposed to injurious concentrations of SO_2 emit H_2S when in light. Because injury by SO_2 is also light-dependent, it was conceivable that H_2S could be a part of the injury mechanism. De Cormis also showed with ^{35}S that most of the sulfur in H_2S came from SO_2 , but some came from sulfate fed to the plants before the injurious SO_2 treatment. We have found that plants emit H_2S in the light when they are fed sulfate without SO_2 being present. The rate of emission can be increased greatly by mechanically injuring the roots prior to immersion in the sulfate solution. Detached leaves treated through the cut petiole also emit H_2S at high rates. The highest rates observed, about $8 \text{ nmoles min}^{-1} (\text{g fresh weight})^{-1}$, are of the same order as expected for the sulfate assimilation pathway of a leaf actively engaged in net protein synthesis. It is therefore possible that H_2S emission is a means by which the plant rids itself of excess inorganic sulfur provided by an active sulfate assimilation pathway. These observations also raise the possibility that living terrestrial plants may be a major source of atmospheric sulfur that has been overlooked previously. About half of the atmospheric sulfur is thought to originate as H_2S , and has been presumed to arise by decay of biological material in anaerobic environments, such as swamps.

The emission of H_2S is not causally related to injury by SO_2 . This was established by finding conditions under which leaves treated with sulfate emitted more H_2S than leaves treated with bisulfite, but only the bisulfite-treated leaves developed SO_2 injury symptoms. These findings do not rule out the possibility that synthesis and emission of H_2S may be a means of detoxifying SO_2 .

The emission of H_2S is light dependent, and responds very rapidly to changes in light intensity. When the light is turned off, a decline in the rate of emission is detected within seconds, and those seconds are probably entirely based on the time that it takes the gas passing the leaf to reach the detector.

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REGULATION OF FORMATION OF PROTEIN COMPLEXES AND ENZYMES IN
HIGHER PLANT CELLS

An obligate coupling of nitrogen and sulfur assimilation exists in plants because the major use made of both elements is synthesis of proteins. Furthermore, although nitrate and sulfate are the usual sources of nitrogen and sulfur, plants can assimilate more reduced forms of both elements. Consequently, nitrate assimilation can be rendered superfluous by the presence of reduced nitrogen or by the absence of sulfur; and sulfate assimilation can be rendered superfluous by the presence of reduced sulfur or the absence of nitrogen. The toxicity of intermediates of nitrate and sulfate reduction necessitates that the intermediates not accumulate excessively. The large amount of energy needed for synthesis of the C-N bond of the α -amino group of amino acids from nitrate also makes it prudent for the plant not to engage in unnecessary reduction of nitrate. All of these considerations point to the need for precisely coordinated regulation of nitrogen and sulfur assimilation in plants. We are continuing our investigations of the mechanisms of regulatory coupling in this system. In the past year, the emphasis has been on the path of ammonia assimilation; the regulation of urease; and selection of variants for resistance to selenium compounds which results from modification of sulfate assimilation.

While changes in the amounts or activities of enzymes are probably responsible for most changes in the rates of metabolic processes, during development organisms also undergo changes in spatial relations: shape, position, internal organization. A great deal of evidence has been amassed in recent years that microtubules play a key role in such changes. Although there have been many ultrastructural studies of microtubules in the plant kingdom, biochemical studies of plant tubulin are rather rare. We are investigating the regulation of microtubule assembly and disassembly during regeneration and shortening of the flagella of *Chlamydomonas reinhardtii*. We are also studying the interactions of proteins of *Colchicum autumnale*, a plant which makes colchicine, an alkaloid which binds to microtubule protein, and prevents microtubule formation in other organisms. We are seeking a higher plant material rich in tubulin.

1. Slow Adaptation of Urease Levels in Tobacco Cells Cultured on Different Nitrogen Sources

T. A. Skokut, P. Filner

Tobacco XD cells which have been subcultured many times in media containing urea as the sole nitrogen source yield extracts having about four times the specific activity of urease as cells which have been subcultured many times in media containing nitrate as the sole nitrogen source. When cells grown on nitrate are subcultured into medium containing urea as the sole nitrogen source, there is little change in specific activity of urease in the first subcultures. However, after repeated subcultures in the medium containing urea, i.e. after many generations in the presence of urea, the specific activity of urease eventually rises. Similarly, when cells which had been maintained in a medium containing urea were subcultured into medium containing nitrate but lacking urea, there was only a very slow decline of specific activity of urease over many generations.

The urease activity, measured after mixing extract from cells with high activity with that from cells with low activity, was additive, indicating that the differences were not caused by the presence of activators or inhibitors of urease. The slow changes in urease activity could conceivably be either selection for variant subpopulations, or slow adaptation of the whole population. The rate of growth of the cells on urea did not change as the specific activity of urease changed in successive subcultures. Consequently, if selection of a subpopulation is occurring, it is in spite of a lack of a strong selective pressure (higher growth rate on urea). It therefore seems likely that the changes in specific activity of urease reflect a slow adaptation of the cells, i.e. a change from one metastable state to another. Because of the analogy to changes in cell states during development, we believe that this phenomenon warrants further study.

2. Selection and Characterization of Tobacco Cell Variants Resistant to Selenoamino Acids

S. Flashman, P. Filner

Work done previously in this laboratory on the cultured tobacco cell system had established that a) the first enzyme of the sulfate assimilation

pathway, ATP sulfurylase, is regulated by an apparent end product repression mechanism, b) the nitrogen assimilation pathway provides a positive ("go") signal which is a prerequisite for derepression of ATP sulfurylase, and c) the sulfate analog, selenate, is most toxic when ATP sulfurylase and presumably the whole sulfate pathway is derepressed (Reuveny, 1977; Reuveny and Filner, 1977). In order to analyze further the regulation of the sulfate pathway and the regulatory coupling between nitrogen assimilation and sulfur assimilation, additional perturbations of the regulatory systems were needed. Selection of variants with altered susceptibility to selenium compounds seemed particularly attractive because the available evidence indicates that selenate is assimilated by the same pathway as sulfate, and the end products, selenocysteine and selenomethionine, are highly toxic to plants. Therefore, selenoanalogs of every step in sulfate assimilation should exist, should be toxic, and hence should be useful for selection of variants with many different perturbations in sulfate assimilation.

Because satisfactory methods do not yet exist for screening non-growing variants of cultured plant cells in the presence of a large excess of normal, growing cells, we were restricted to selection for growing cells against a background of non-growing cells, i.e. selection for resistance to toxic seleno compounds. Resistance to selenocysteine or selenomethionine probably could be achieved most readily by excluding the amino acid from the cell. However, simultaneous resistance to both selenoamino acids seemed unlikely to be achieved by exclusion because of evidence that L-cysteine and L-methionine are taken up by separate transport systems, the one for L-cysteine but not the one for L-methionine being inhibitable by L-arginine, (Hart and Filner, 1969). Resistance to both selenoamino acids could be achieved in cells growing on sulfate by derepression of the sulfate pathway, which is normally repressed during growth on sulfate. We undertook selections for resistance to selenocysteine and selenomethionine in the hope of obtaining variants in which the sulfate pathway was permanently derepressed.

Three variants were obtained by selection for growth in the presence of 3 μ M each of selenocystine and selenomethionine, and have been given the designations SCM 4, SCM 7 and SCM 28. All three were found on retesting to have resistance to selenocystine, but only SCM 7 gave indications of

resistance to selenomethionine. Further characterization work was concentrated on SCM 7.

When grown on L-cysteine as the sole sulfur source, ATP sulfurylase of the parent cell line (XDR^{thr}) is repressed, and so the XDR^{thr} cells are relatively resistant to selenate. The SCM 7 variant is more sensitive to selenate than the XDR^{thr} cell line when grown on L-cysteine; this finding is indicative of the sulfate pathway being derepressed in SCM 7. Direct measurements of ATP sulfurylase have indicated the presence of 2-4 times as much ATP sulfurylase activity in SCM 7 cells as in XDR^{thr} cells.

The SCM 7 cells are not more resistant to selenomethionine alone than XDR^{thr} cells. However, SCM 7 cells are more resistant to combinations of selenocystine and selenomethionine than can be accounted for by their resistance to selenocystine alone.

In summary, selection in cultured tobacco cells for simultaneous resistance to selenocystine and selenomethionine yields variants with perturbations in the regulation of sulfur metabolism.

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3. Studies with ^{13}N on the Paths of Nitrogen Assimilation in Cultured Tobacco Cells

T. Skokut, C. P. Wolk, P. Filner

Two enzymatic routes have been proposed for the assimilation of ammonia in higher plants: (i) synthesis of glutamate from ammonia and α -ketoglutarate which is catalyzed by glutamic dehydrogenase; followed by transaminations, including synthesis of glutamine; (ii) synthesis of glutamine from ammonia and glutamate, which is catalyzed by glutamine synthetase, followed by synthesis of two molecules of glutamate from glutamine and α -ketoglutarate, which is catalyzed by glutamate synthase. One glutamate would be reused for ammonia fixation and the other would be used in transaminations. These two alternatives lead to opposite predictions about the temporal sequence of labeling of glutamate and glutamine with isotopic nitrogen. Thomas et al. (1977) have developed procedures for preparing $[^{13}N]NH_3$, feeding the labeled compound to algae, then extracting and analyzing the amino acids to determine the labeling pattern in spite of the 10 minute half-life of the isotope.

These procedures have been applied to cultured tobacco cells grown on nitrate, ammonia or urea.

In all media tested, the tobacco cells incorporate [^{13}N] NH_3 into glutamine much more rapidly than into glutamate. Methionine sulfoximine, an inhibitor of glutamine synthetase, inhibits incorporation of [^{13}N] NH_3 into both glutamine and glutamate in the tobacco cells; azaserine, an inhibitor of glutamate synthase, inhibits incorporation of [^{13}N] NH_3 into glutamate but not glutamine in these cells. These results strongly support the hypothesis that ammonia is assimilated via the glutamine synthetase-glutamate synthase pathway in cultured tobacco cells grown on nitrate.

Numerous attempts have been made to confirm the above interpretation by performing pulse-chase experiments, using addition of excess unlabeled ammonia 10 - 120 seconds after introduction of [^{13}N] NH_3 to achieve the pulse and chase. Although the radioactivity could be chased out of [^{13}N]glutamine very effectively (better than 90% was chased 2 minutes after starting the chase), the radioactivity did not reappear in glutamate, nor in other soluble organic compounds. Although the reason for this anomalous result is not entirely clear, back exchange of ^{13}N from glutamine to ammonia seems the most plausible explanation.

At present, methodology for doing similar experiments with [^{13}N]nitrate is under development.

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4. Herbicides with Anti-mitotic Activity: Potential Tools for Studying Microtubules in the Plant Kingdom

H. Quader, P. Filner

Colchicine, an antimitotic drug which disrupts microtubules and binds to the microtubule protein, tubulin, is widely used to assay for tubulin from animal sources. In contrast to the situation in animal systems, where 1 μM is often effective, more than 1 mM is usually required to elicit an effect in algal and plant systems. Furthermore, only very low levels of colchicine binding have been detected so far in nitro in these systems,

which brings into question the usefulness of colchicine binding for identifying tubulin from those sources. Tubulin from plants and algae may have an inherently low colchicine binding activity, or the binding reaction may be subject to interference in plant extracts. Extracts of the alga *Ochromonas* inhibit the colchicine binding activity of cow brain tubulin. Extracts of tobacco plants are also inhibitory (N. Yadav, unpublished data). The herbicide, trifluralin, has been reported to block mitosis and cause microtubules to disappear (Hess and Bayer, 1974). Oryzalin (Eli Lilly) is another herbicide reported to possess antimitotic activity (Bartels and Hilton, 1973). Dr. Carl Fedtke of Bayer brought to our attention still another herbicide, under development by Bayer and called amiprofosmethyl, with antimitotic activity. Amiprofosmethyl, trifluralin and oryzalin block cell division at concentrations as low as 1 μ M. If the action of an herbicide were similar to that of colchicine, it would be useful for solving the problem of identification of plant and algae tubulin. To elucidate the action of these herbicides we chose to investigate their effects of flagellar regeneration in *Chlamydomonas* and compare their effects with that of colchicine.

About 2 mM colchicine is necessary to prevent flagellar regeneration in *Chlamydomonas*, whereas the herbicides inhibit regeneration at as little as 3 - 5 μ M. When colchicine is added after regeneration has started, further flagellar outgrowth is stopped but the portion already regenerated persists. However, when an herbicide is added after regeneration has started, the portion already regenerated begins to shorten. Colchicine at 10 mM has no effect on the length of flagella when added after regeneration is complete, but 10 μ M of an herbicide causes the cells to shorten their flagella almost completely in 90-120 minutes. Amiprofosmethyl appears to be the most effective of the three herbicides tested. However, this finding may be based on the greater water solubility of amiprofosmethyl compared to trifluralin and oryzalin. (Solutions of these compounds are made by dissolving them in acetone and diluting, 1:100, into water). In contrast to the flagellar shortening caused by EGTA, that caused by the herbicides is not reversible by Ca^{2+} . Experiments are in progress with [^{14}C]amiprofosmethyl to characterize a protein fraction of *Chlamydomonas* to which it binds. The fungicide, benziimidazol-2-yl carbamate, has been reported to bind to a fungal tubulin which has very little colchicine binding activity (Davidse and Flach, 1977).

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5. Chemically Induced Flagellar Shortening in *Chlamydomonas reinhardtii*

H. Quader, J. Cherniack, P. Filner

Flagellar "retraction" or "resorption" (a more neutral term would be "shortening") and regeneration are normal processes in the life cycle of *Chlamydomonas reinhardtii*. The regeneration process has been studied in the past because it could be induced experimentally. Recently, experimental means of inducing flagellar shortening in whole populations of *Chlamydomonas* have been described. Exposure to pyrophosphate (LeFebvre, 1976) or to caffeine (Hartfield and Amrhein, 1976) causes flagellar shortening. A temperature-sensitive motility mutant has been described in which the flagella shorten at the restrictive temperature (Huang et al., 1977).

We have found that in addition to pyrophosphate, the tetraacids EGTA and EDTA will also induce flagellar shortening, while mono-, di- and tri-carboxylic acids have little or no activity. Because all of the active molecules are good chelators of divalent cations, and EGTA has a relatively high affinity for Ca^{2+} compared to Mg^{2+} , we investigated the role of Ca^{2+} in flagellar shortening and regeneration. Ca^{2+} , Sr^{2+} and Mn^{2+} were equally effective at causing regeneration after EGTA-induced shortening, while Ba^{2+} and Mg^{2+} were less effective. Regeneration after removal of flagella by pH shock could be inhibited by as little as 100 μM EGTA, while more than 1 mM EGTA was required to initiate flagellar shortening. Only micromolar amounts of free Ca^{2+} are required to overcome the inhibition of regeneration by 100 μM EGTA, while Mg^{2+} was much less effective at a concentration 100x higher.

These results indicate that Ca^{2+} has a key role in flagellar shortening and regeneration. The other divalent cations may cause regeneration after EGTA-induced shortening by displacing bound Ca^{2+} and thereby elevating the free Ca^{2+} concentration.

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6. Fate of Flagellar Proteins During Shortening of Flagella of *Chlamydomonas reinhardtii*

A. Handa, J. Cherniack, P. Filner

The experiments of Coyne and Rosenbaum (1970), showing that when one flagellum of *Chlamydomonas* is sheared off, the other shortens as the lost one is regenerated, were highly indicative of the idea that *Chlamydomonas* resorbs flagellar constituents during shortening and reutilizes them. Until recently, however, no experimental method existed whereby all of the cells in a population could be induced to shorten their flagella, so there was no opportunity to subject the "resorption" hypothesis to a biochemical test. Using pyrophosphate to cause shortening, and $^{35}\text{SO}_4^{2-}$ to radiolabel flagellar proteins, we have examined the fate of flagellar proteins during shortening. The ^{35}S in flagellar proteins, assayed as the radioactivity precipitable from the flagellar fraction by trichloroacetic acid, disappeared from the flagellar fraction in parallel with flagellar shortening. There was a concomitant appearance of about the same amount of acid precipitable radioactivity in the soluble fraction of the medium. However, the appearance of acid precipitable radioactivity in the medium continued even after shortening was completed. When radioautograms were made from SDS-polyacrylamide gels used to analyze electrophoretically the radioactivity released into the medium, the two polypeptides characteristic of flagellar tubulin produced the major bands.

We conclude that the major fate of flagellar proteins, including tubulin, during shortening is released into the medium. After completion of flagellar shortening, the cells continue to release the same proteins, probably in a futile attempt to regenerate flagella.

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7. Release of a Phosphorylated Compound from *Chlamydomonas reinhardtii* during Flagellar Shortening

J. Cherniack, A. Handa, R. A. Bressan, P. Filner

When *Chlamydomonas reinhardtii* cells, prelabeled by growth in medium containing [^{32}P]inorganic phosphate, shorten their flagella in response to exposure to pyrophosphate, radioactivity is released into the medium. Unlabeled phosphate does not cause shortening or release of the radioactivity. The radioactivity can be separated from inorganic phosphate and pyrophosphate by thin layer or ion exchange chromatography, or by electrophoresis. The radioactivity behaves as a single compound in 2-dimensional chromatography. The compound can also be released from isolated flagella by treatment with pyrophosphate. The cells do not release the compound in the absence of flagella. The compound is acid labile, and breaks down into at least two kinds of phosphorus-containing moieties. Based on chromatographic comparisons, as well as ultraviolet spectrophotometry, the compound is not one of the common nucleotides. Our present goal is to prepare it in pure form and to determine its structure.

The available evidence indicates that the compound is a flagellar constituent or is derived from a flagellar constituent. It is the only low molecular weight compound containing phosphate which accumulates in the medium during flagellar shortening. Tubulin is known to have bound guanine nucleotides. However, they have been ruled out by chromatographic comparisons with the unknown compound.

THE PRIMARY CELL WALL AND SOLUBLE HYDROXYPROLINE-RICH GLYCOPROTEINS

Our interest in the plant cell wall derives from the consideration that it is both an instrument and a result of morphogenesis. We postulate that the primary cell wall is not an amorphous mass of cellulose, pectins and protein but a proteoglycan network in which a hydroxyproline-rich glycoprotein cross-links the cell wall polysaccharides. Cleavage of critical covalent linkages in this network would result in increased cell wall plasticity; hence the name "extensin" for the hydroxyproline-rich glycoprotein present in the primary cell wall of most green plants.

Our current work is devoted to determination of the structure of extensin released from the cell walls of in vitro cultured cells of higher plants. We have demonstrated two linkages by which extensin may be linked to cell wall polysaccharides: arabinosyl hydroxyproline and galactosyl serine. In order to define the glycosidic linkages to extensin further, and to determine the amino acid sequence of extensin, we are attempting to release large fragments of extensin from the cell wall by β -elimination under semi-aqueous alkaline conditions and by sodium chlorite oxidation of depectinized cell walls.

Last year we demonstrated a new class of hydroxyproline-rich glycoproteins which differ from extensin in being rich in alanine and having the linkage galactosyl hydroxyproline. A subsequent report indicates the presence of hydroxyproline-alanine-rich glycoproteins in seeds (Jermyn and Yeow, 1975). Upon the premise that knowledge of structure may give insight into function, we continue our work on the purification and characterization of soluble hydroxyproline-rich glycoproteins secreted into the media of tomato and sycamore-maple suspension cultures.

Reference

Jermyn, M. A. and Y. M. Yeow, Austr. J. Plant Phys. 2:501 (1975)

1. New Methods for the Extraction of Cell Wall Protein

(a) *Via β -Elimination under Semiaqueous Conditions*

D.T.A. Lamport, M. Caughey

This work continues directly that described last year, namely, chemical characterization of hydroxyproline(hyp)-rich material released from tomato cell walls under conditions designed to bring about β -elimination of serine residues (dimethyl sulfoxide, water, methanol 50:40:10, v/v, containing 0.2 N NaOH and 1 M NaBH₄ at 45°C for 5 hrs. Our main advance this year has been effective fractionation of the "low molecular weight" glycopeptides (5,000-10,000 daltons), aided by the observation that the low molecular weight glycopeptides are freely soluble in 80% ethanol. Further fractionation of these low molecular weight glycopeptides on the cation exchanger sulfopropyl Sephadex and the anion exchanger DE-52 (DEAE cellulose) yielded five major fractions differing mainly in their lysine and carbohydrate content, and forming a series of increasingly basic glycopeptides. The least basic of these glycopeptide fractions is currently of greatest interest to us, because its serine/galactose ratio is much greater than one (actually around 5 or 6) and therefore an indication of oligosaccharide attachment, and also because we have for the first time observed a major fraction which also contains large amounts of galacturonic acid (ca. 20 residues/serine). If verified by further analysis this will be an important step in confirming the suggestion that extensin cross-links polysaccharides via the serine residues.

(b) *Further Characterization of NaClO₂ Released Peptides*

D.T.A. Lamport, A. Mort

Peptides released from tomato cell walls by NaClO₂ oxidation band around a density of 1.51 in a CsCl gradient. As the density of non-glycosylated proteins is usually about 1.3 and that of polysaccharides 1.6 this indicates that the peptides are highly glycosylated. Peptides purified in this way contain arabinose, galactose, glucose and small amounts of galacturonic acid. Methylation analysis of these peptides shows them to contain arabinosides, as previously described, and a terminal galactose residue on approximately 80% of the serine hydroxyl group. There are also smaller amounts of 1,4-linked galactose and glucose, and what appears to be 3,6-linked galactose, indicating that the proposal put forward by

Keegstra et al. (1973) that extensin is linked to the wall matrix via a 3,6-linked arabinogalactan, may be correct.

Reference

Keegstra, K., K. W. Talmadge, W. D. Bauer and P. Albersheim,
Plant Physiol. 51:188 (1973)

2. Soluble Hydroxyproline-Rich Glycoproteins from Higher Plants

D.T.A. Lamport, J. A. Clarke

We continued to purify soluble hyp-rich glycoproteins secreted into the medium of sycamore-maple and tomato suspension cultures. We initially concentrated the glycoprotein by ultrafiltration of the medium from 12 day old cultures. After desalting on Sephadex G-25, the material retained by the ultrafilter membrane is lyophilized and subjected to HF (hydrogen fluoride) deglycosylation. Before HF treatment the glycoprotein voids a Sephadex G-100 column; after HF treatment the deglycosylated hydroxyproline-rich protein is retarded on G-100 and contains less than 10% of the sugars present in the crude preparation. Based on amino acid analysis before and after HF treatment, we have achieved a twofold enrichment in hydroxyproline. The carbohydrates associated with the hydroxyproline-rich fraction after HF treatment are arabinose, galactose and glucose in the molar ratio 1:1:1. Hydrazinolysis of the HF-treated glycoprotein results in a loss of 30% of the serine residues in the case of the glycoproteins from sycamore cells and a loss of 54 percent of the threonine residues and 32% of the serine residues from the glycoprotein from tomato cells. This indicates that some of the carbohydrate residues associated with the protein after HF treatment are probably linked to serine and threonine.

We attempted to purify the HF-treated glycoprotein further by isoelectric focussing, and after this separated an acidic fraction from a hyp-alanine-rich fraction.

The amino acid profiles of these partially purified extracellular glycoproteins from tomato and sycamore-maple cells are similar to that reported for another extracellular glycoprotein from tobacco cells, to a glycoprotein found in sugar maple sap, and to the profiles of hyp-rich seed proteins. All these glycoproteins are rich in hyp, serine and alanine, indicating that these glycoproteins are highly conserved from an evolutionary point of view.

3. Preliminary Analysis of Dinoflagellate Cell Walls

J. Kemerer, J. A. Clarke

Important to tracing the evolution of extensin is the knowledge of whether extensin-like glycoproteins are present in primitive photosynthetic eukaryotes. We chose to analyse the cell walls of dinoflagellates as these organisms are readily available representatives of these eukaryotes. Our first project was to determine whether the cell walls contain hydroxyproline.

We cultured Amphidinium spp. on Erdschreiber-sea water medium, harvested the cells by centrifugation, and isolated the cell walls by sonication followed by repeated washing with 0.5 NaCl to eliminate cytoplasmic contamination. After washing with water to remove the salt from the wall, amino acid analyses of this cell wall preparation were carried out. The results showed that the cell walls of these dinoflagellates contain 12.5 μg hyp/100 mg; this is low when compared with the range of 50 μg to 2.7 mg/100 mg for the cell walls of plants so far studied, i.e. from the Chlorophyceae upwards.

4. The Effect of HF on Glycoproteins

A. Mort, D.T.A. Lamport

It is now established, from work with cell wall peptides, fetuin, pig submaxillary mucin and bovine serum albumin, that anhydrous HF at 0°C, in the presence of anisole, will cleave almost quantitatively the glycosidic linkages of neutral sugars, without breaking peptide bonds or modifying any of the amino acids present. The HF treatment can be used to assist in purifying and characterizing heavily glycosylated proteins. As the glycosidic linkages of amino sugars are not cleaved under these conditions amino sugars linked directly to proteins will not be removed; this should facilitate the determination of glycopeptide linkages.

At 23°C the O-glycosidic linkages of amino sugars are cleaved within 3 hrs thus allowing almost complete (ca. 98%) deglycosylation of pig submaxillary mucin. A 23°C treatment of fetuin leaves only N-acetyl glucosamine linked to the protein via the N-glycosidic-asparaginyl linkages.

MOLECULAR STUDIES OF MEMBRANES AND THE MECHANISMS OF
CELLULAR ADAPTATION TO THE ENVIRONMENT

We continue to investigate certain physico-biochemical properties of biological membranes, and the role these membranes may have in the adaptation of cells of both pro- and eucaryotic organisms to a particular environment. We are therefore, on one hand, continuing investigations of the membranes of simple mycoplasmas in order to obtain insight into the extent to which membranes are capable to interface with their environment. With these microbial membranes we had shown that the membrane lipid fluidity is kept virtually constant under different environmental conditions such as changes in growth temperature and nutrient supply. This principle may also apply to eucaryotes. To test this point of view we are now, on the other hand, also investigating adaptive differentiation of an aquatic fungus, *Blastocladiella emersonii*, an organism that appears to be highly suitable for investigating adaptive mechanisms during differentiation since it possess four distinct developmental pathways which are determined by environmental factors (e.g., temperature, ions). Adaptive membrane alterations in response to environmental salt stress are also investigated in two tomato varieties, a salt tolerant and a salt sensitive one.

As in previous years we continue to study fast photoprocesses in the photosynthetic light transducer. To investigate such photoprocesses, the emission of delayed light is recorded after turning off the exciting light.

1. Membrane potential of *Thermoplasma acidophilum*

J. C. Hsung, A. Haug

Thermoplasma acidophilum is a mycoplasma-like microorganism which grows optimally at a temperature of 59°C and a pH of 2. We demonstrated previously that the intracellular pH of this organism is close to neutral. Furthermore, the pH gradient is maintained passively by a Donnan equilibrium. Now we were able to show that part of the pH gradient is maintained by a membrane potential.

The potential was measured by the distribution of radioactive KS^{14}CN , which almost completely dissociates at $\text{pH} \geq 2$. In addition, a radioactive lipophilic cation, tetraethylammonium (TEA^+), was used to determine the polarity of the cell. The SCN^- anion was accumulated in the cell whereas the TEA^+ cation was not. This result demonstrates that the cells are positive inside. The membrane potential lies between 109 and 125 mV. The question has now to be raised whether the chemiosmotic theory of energy coupling, proposed by P. Mitchell, can be applied to this organism, i.e., can the respiratory transport of H^+ and the return of H^+ to induce ATP synthesis be the mechanism for energy transduction in *Thermoplasma acidophilum*? In this organism the huge pH gradient is partially balanced by the membrane potential, positive inside. The net electrochemical gradient in our microorganism at the resting state amounts to about 170 mV, i.e., 290 mV at 56°C for the pH gradient minus 120 mV for the Donnan potential. Is the value of 170 mV incompatible with the Mitchell hypothesis? The answer is negative since the electrochemical potential of about 300 mV required the ATP synthesis is not the net electrochemical potential but rather the proton motive force in the energized state. Obviously our investigation does not provide any direct information in support of the chemiosmotic theory either. Nevertheless, Mitchell's hypothesis offers an explanation for the fact that *Thermoplasma acidophilum* does not grow above pH 4.5, since at this pH value the membrane potential ψ drops to + 40 mV. Consequently, the relation $|\psi - (RT/F)\ln(\Delta\text{pH})|$ (negative inside), becomes less than 80 mV. This makes the coupling mechanism non-functional. Thus the cell is not shielded passively from the acidic environment.

2. ζ -Potential and Surface Charge of *Thermoplasma acidophilum*

J. C. Hsung, A. Haug

The surface charge density and the ζ -potential of *Thermoplasma acidophilum* was estimated from microscopic electrophoresis experiments. The surface charge is negative. This conclusion is based on several observations: (a) The cells moved from the negative electrode to the positive one. (b) When the external pH was raised above pH 6, the mobility increased. This resulted presumably from the fact that more

negative charges were exposed by raising the pH. (c) Ca^{2+} ions, in the concentration range of 1 mM and higher, reduced the electrophoretic mobility at pH 6. Ca^{2+} ions are specifically bound to the negatively charged cell surface, probably to the phosphate head groups of the phospholipids.

In a medium with an ionic strength close to that of the growth medium, pH 2, the surface charge density is 1360 esu cm^{-2} . This charge density corresponds to 5.7×10^4 elementary charges per cell or about one elementary charge per $3,500 \text{ \AA}^2$.

At pH 2 and an ionic strength similar to that of the growth medium, the ζ -potential was about 8 mV, negative relative to the bulk medium. The ζ -potential represents the potential difference from the cell surface of shear relative to the bulk medium. We do not have any information about the surface on the cytoplasmic side of the membrane. It may be positive or negative. Thus a ζ -potential may also exist on the inner surface of the membrane. In Figure 1, this possible ζ -potential is tentatively drawn as positive.

Fig. 1. A model for *Thermoplasma acidophila*; the surface is negatively charged, and the potential difference between two bulk phases is 120 mV (positive inside), a zeta-potential of about 8 mV exists between the external surface of shear to the bulk phase of the external medium, when the external medium is at the same pH, ionic strength and divalent ion concentrations of the growth medium.

3. Membrane Structure of a Thermophilic, Acidophilic Mycoplasma

L. L. Yang, A. Haug

Previously we had found that *Thermoplasma acidophilum*, which grows at 56°C and pH 2, has a highly rigid membrane lipid matrix which allows the organism to grow in such a harsh environment. Therefore, we continued research on these membranes in order to understand the evolutionary limits of adaptation and the underlying structural membrane components. The lipids of this protein-rich (75%, w/w) membrane were studied by thin layer chromatography. The ratio of neutral lipid/glycolipid/phospholipid was 1:1:3 (w/w), with one phospholipid band accounting for 50% of the total lipids. Gas chromatographic and mass spectroscopic investigations of the lipids showed the presence of large quantities of long-chain alkyl glyceryl ethers, but very few fatty acid esters. Glycolipids comprised predominantly (70%, w/w) monoglycosyl ether linked diglycerides. Highly charged head groups were found in 80% of the phospholipids. In the membrane of *Thermoplasma acidophilum* we discovered glycoproteins by gel electrophoresis. Since no thorough study is available regarding glycoproteins from procaryotic membranes, we intend to determine accurately the carbohydrate-protein linkage. Moreover, we wish to ascertain whether these membrane surface carbohydrates play any role in adaptive protection towards the harsh environment.

4. Adaptive Differentiation in *Blastocladiella emersonii*

K. Leonards, J. Jen, A. Haug

Since our knowledge of the environmental and developmental limits of adaptation is rather limited and scattered, we are attempting to obtain information on causal relationships between ecological conditions and physico-chemical responses of basic cell functions at the membrane level. In order to investigate adaptive differentiation and associated structural and molecular mechanisms involved therein, we study the aquatic fungus *Blastocladiella emersonii* which, for reasons already mentioned, seems to be highly suitable for studying adaptive processes during differentiation.

During the past year we succeeded to culture these cells with yields of 10^{11} zoospores per harvest. Moreover, a procedure has been worked out for the isolation of zoospore plasma membranes. Chemical, cytochemical and microscopic tests indicate that these isolated membranes are highly purified; e.g., they contain less than 0.3% nucleic acid contamination as determined by the orcinol procedure. Now we are in a position to perform physico-biochemical experiments concerning adaptive responses as a function of external conditions.

We found that the membrane surface of zoospores binds surface active compounds such as concanavalin A which attaches to surface carbohydrates. If we are able to determine the number of surface binding sites, that number can be used as a parameter to study adaptive responses of membrane constituents exposed to the environment.

5. Adaptation of Tomato Root Cell Membranes to Saline Stress

C. Caldwell, A. Haug

We are investigating two tomato varieties, a salt tolerant and a salt sensitive one. We wish to ascertain whether adaptive membrane alterations occur at the physico-biochemical level in response to saline stress. Specifically we would like to answer the question whether the cell membrane constitutes the primary stress sensor in plants which are exposed to external stresses.

Presently we are trying to isolate plasma membranes from halo-tolerant and halosensitive tomato varieties. In comparative studies these two types of membranes will be analyzed biochemically for the lipid and protein composition, properties of membrane-bound enzymes and for their membrane lipid fluidity.

6. Stimulation of Microsecond Delayed Fluorescence in Spinach Chloroplasts by Uncouplers

D. Bell, N. Good (MSU Department of Botany and Plant Physiology), A. Haug

Delayed fluorescence of chloroplasts is thought to be a back reaction of the reduced primary electron acceptor of photosystem II (Q^-) and the oxidized primary electron donor of the photosystem (Z^+). Uncouplers

inhibit that portion of delayed fluorescence which is emitted milliseconds after turning off the excitation light; this can be related to the inhibition of the formation of the membrane potential. When measurements were made a few microseconds after light extinction, the intensity of delayed light emission increased in the presence of uncouplers. Moreover, the rate of decay of the delayed light was increased by the uncouplers which leads eventually to the already known inhibition of millisecond delayed light. Simultaneous measurements of microsecond delayed light and electron transport indicate that uncouplers act by increasing the rate of electron transport, thus altering the levels of Q^- and Z^+ . Therefore, it is perhaps not necessary to invoke changes in the membrane potential in order to explain the effects of uncouplers on delayed light emission.

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