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TRANSACTIONS OF THE THIRD
COMMISSION OF THE INTERNA-
TIONAL SOCIETY OF SOIL SCIENCE

COMPTES RENDUS DE LA TROISIÈME
COMMISSION DE L'ASSOCIATION IN-
TERNATIONAL DE LA SCIENCE DU SOL

VERHANDLUNGEN DER DRITTEN
KOMMISSION DER INTERNATIONALEN
BODENKUNDLICHEN GESELLSCHAFT

AUGUST 30 - SEPTEMBER 1, 1939

VOLUME B

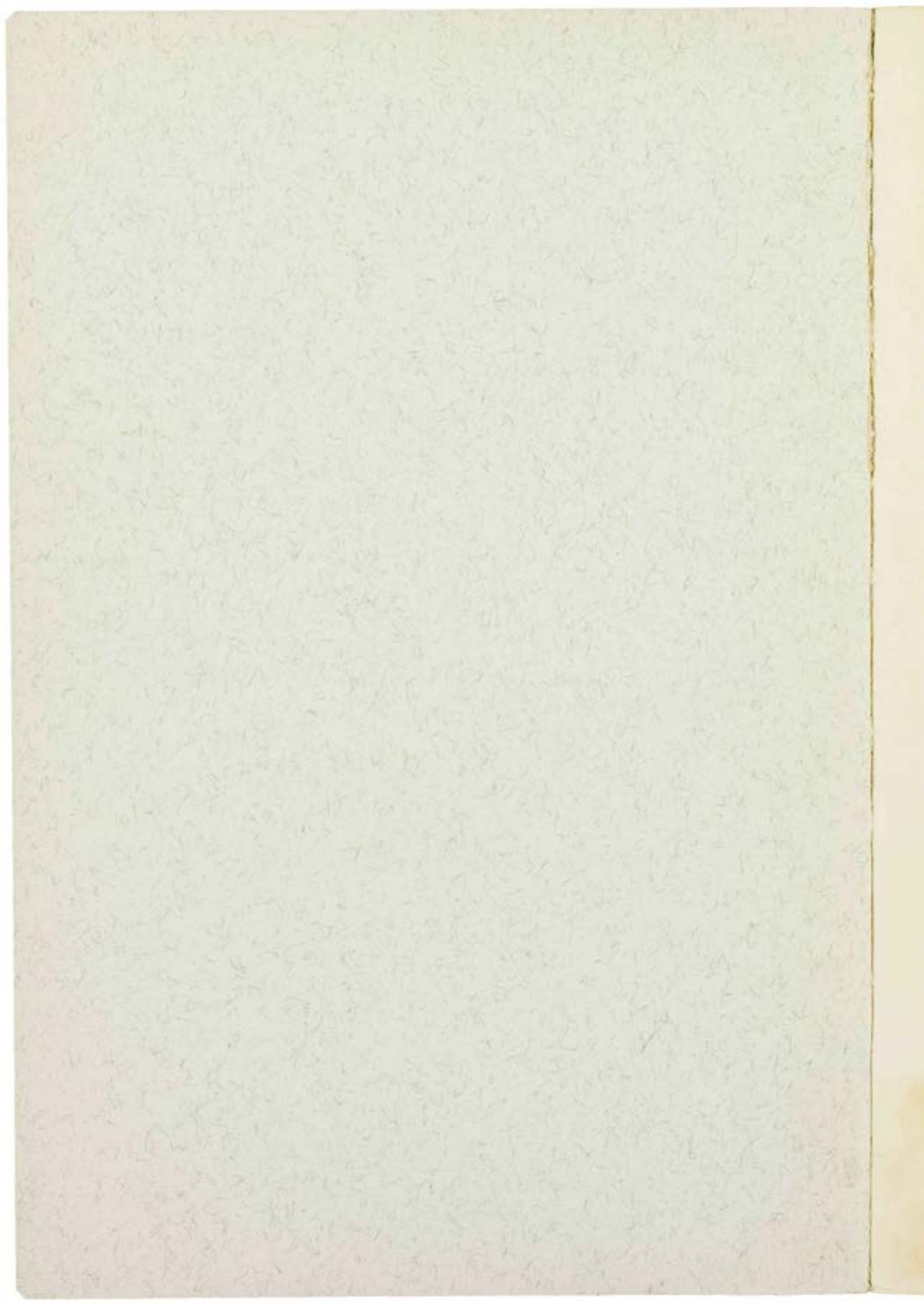
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Page 2



PROCEEDINGS OF THE THIRD COMMISSION
OF THE INTERNATIONAL SOCIETY OF SOIL SCIENCE



Photo by G. H. Pound

MEMBERS OF THE CONFERENCE OF THE THIRD COMMISSION

Front Row, left to right: D. W. Thorne, S. C. Vandecaveye, T. C. Peele, A. G. McCall, N. Thomas, J. B. Hester, B. Wolf, H. C. Knoblauch, D. Q. Anderson, A. W. Peirce, W. B. Sarles, W. W. Umbreit, C. E. Skinner, E. O. Karow, J. P. Sell, 2nd Row: J. Smit, S. A. Waksman, O. Schreiner, D. Burk, E. B. Fred, Mrs. R. L. Starkey, J. K. Wilson, J. Dufrenoy, Mrs. S. A. Waksman, F. Chodat, C. Thom, H. G. Thorton, H. J. Conn, H. W. Batchelor, H. W. Reuszer, 3rd Row: L. W. Erdman, W. A. Albrecht, T. L. Martin, J. P. Martin, T. P. Martin, W. P. Martin, A. Wilson, W. V. Halversen, J. L. Stokes, T. C. Cordon, R. E. Buchanan, C. Stapp, J. F. Reed, Miss M. R. Greene, S. F. Snieszko, F. Scheffer, M. F. Spaulding, 4th Row: I. H. Baldwin, F. E. Allison, A. A. Hendrickson, L. T. Leonard, C. A. Ludwig, E. J. Mulder, H. A. Barker, H. B. Woodruff, H. Katznelson, K. V. Thimann, P. H. H. Gray, O. Wyss, R. K. Tam, R. H. Burris, Mrs. E. K. Allen, R. M. Stern, H. A. Lunt, A. G. Norman, O. N. Allen, C. E. Zohell, J. S. Joffe, F. D. Richardson, F. Giesecke, R. Bradfield, H. L. Bodily, J. F. Marrero, *Rear Group at right*: A. G. Lochhead, F. B. Smith, T. Gibson, K. L. Jones, R. L. Starkey, M. F. Morgan, F. S. Orcutt, Norman James, N. R. Smith, F. E. Clark, M. I. Timonin, A. Schenkman, H. C. Lint, I. J. Hutchings, J. W. Foster.

COMMISSION III OF THE INTERNATIONAL
SOCIETY OF SOIL SCIENCE

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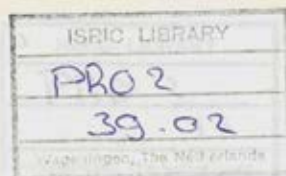
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1079 B

PROGRAM COMMITTEE

E. B. FRED, in charge of Legume program.

CHARLES THOM, in charge of Organic Matter program.

A. W. HOFER, representing Soil Science Society of America.

SELMAN A. WAKSMAN, *Chairman*

LOCAL COMMITTEE ON ARRANGEMENTS

J. A. ANDERSON

E. W. MORRISON

R. L. STARKEY, *Chairman*

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PREFACE

THE meetings of the Third Commission of the International Society of Soil Science were held, as scheduled, from August 30 through September 1. Many delegates began to assemble on Tuesday, the day preceding the meeting, and a number remained during Saturday morning, following the meeting. In all, some 120 scientific workers took part. Many were accompanied by their wives and other members of their families.

The organizing committee takes this opportunity to thank again those organizations, listed in volume A, whose contributions made this meeting possible. Thanks are also extended to Dr. W. H. Martin, Director of the New Jersey Agricultural Experiment Station and Dean of the College of Agriculture of Rutgers University, for helpful assistance and encouragement in arranging and conducting the meeting; to the authorities of the New Jersey College for Women, for permission to use The Lodge as a meeting place and for arranging the dormitory facilities; to the New Jersey State Chamber of Commerce and the New Jersey State Department of Agriculture, for supplying various publications for free distribution; and to the authorities of The Squibb Institute, Princeton University, the Rockefeller Institute at Princeton, the Walker-Gordon Laboratories, the New Jersey State Department of Agriculture, the Cranberry Substation and Miss White at Whitesbog, for numerous courtesies in connection with the visits and excursion. The banquet on Thursday night was given gratis by the College of Agriculture of Rutgers University. The luncheon on Friday was provided through the generosity of the Walker-Gordon Laboratories.

The organizing committee wishes to express further its appreciation to the Soil Science Society of America for contributing the necessary funds to publish volume B and to Dr. T. M. Rivers, President of the Third International Congress for Microbiology, as well as to the organizing committee of the Congress, for collaborating in various ways and in facilitating the organization of these meetings.

In addition, the chairman wishes to express his appreciation to the many other persons who contributed to the success of the meetings through active participation in the program and in arranging accommodations for those who attended the meetings.

PROGRAM

WEDNESDAY, AUGUST 30

MORNING

Registration in Administration Building, N. J. College of Agriculture.
Visits to exhibits in Administration Building, to laboratories, and to field experiments.

AFTERNOON

Meeting at The Lodge, N. J. College for Women—1:30 o'clock.
Welcome by Dr. W. H. Martin, Director of the N. J. Agricultural Experiment Station and Dean of the College of Agriculture.
Scientific Program—*Legumes and Legume Bacteria*.

EVENING

Grilled steak picnic supper—Log Cabin on Horticulture Farm.

THURSDAY, AUGUST 31

MORNING

Meeting at The Lodge—9:00 o'clock.
Program—*Microbiology of Soil Organic Matter*.
Official photograph at The Lodge.
Box luncheon at the Dairy Building.

AFTERNOON

Visit to laboratories of Squibb Research Institute.
Meeting at The Lodge—2:00 o'clock.
Program—*Azotobacter and Its Significance in Soil Processes*.
Business meeting.
Round table discussion—*Legume Inoculants*, led by A. W. Hofer.
Tea served at the home of Mrs. W. H. Martin, wife of the Director.

EVENING

Complimentary banquet by the College of Agriculture, Rutgers University,
Hotel Roger Smith—7:30 o'clock.

FRIDAY, SEPTEMBER 1

EXCURSION. Departure from New Brunswick at 9:00 o'clock.

MORNING VISITS

Princeton University

Rockefeller Institute for Medical Research. Laboratories for animal and plant diseases.

Walker-Gordon Laboratories. Visit to Rotolactor, barns, and drier.

Buffet luncheon served with the compliments of the Company.

AFTERNOON VISITS

Nematode laboratory of N. J. State Department of Agriculture—White Horse.

Farm demonstrating Soil Conservation practices.

Substation of N. J. Agricultural Experiment Station for research on cranberries and blueberries—Whitesbog.

Asbury Park shore resort.

SATURDAY, SEPTEMBER 2

MORNING—Optional tours, starting at 9:00 o'clock.

1. Visits to laboratories, exhibits, and field experiments. Tour of experiments on plant physiology; field crops and turf (Agronomy department); soil fertility plots, cylinders, lysimeter experiments (Soils department); experiments on fruits and vegetables (Horticulture department).
2. Visit to Soil Conservation Station at Marlboro and to demonstration farms of Soil Conservation Service.
3. Tour of campus of Rutgers University.

LIST OF PARTICIPANTS

AUSTRALIA

A. W. Peirce, Waite Agricultural Research Institute, Adelaide, South Australia.

CANADA

V. E. Graham, University of Saskatchewan, Saskatoon.
P. H. H. Gray, Macdonald College, Quebec.
Norman James, University of Manitoba, Winnipeg.
A. G. Lochhead, Dominion Department of Agriculture, Ottawa.
N. J. Thomas, Ontario Agricultural College, Guelph.
M. I. Timonin, Dominion Department of Agriculture, Ottawa.

ENGLAND

H. G. Thornton, Rothamsted Experimental Station, Harpenden.

FRANCE

J. Dufrenoy, Faculté des Sciences, Bordeaux.

GERMANY

F. Giesecke, University of Berlin, Berlin.
F. Scheffer, Landwirtschaftliches Chemisches Institut, Jena.
C. Stapp, Biologische Reichsanstalt, Berlin-Dahlem.

HOLLAND

E. G. Mulder, Agricultural University, Wageningen.
Jan Smit, Agricultural University, Wageningen.

POLAND

S. F. Śnieszko, University of Poland, Cracow.

SCOTLAND

T. Gibson, College of Agriculture, Edinburgh.

SWITZERLAND

F. Chodat, Geneva University, Geneva.

UNITED STATES

Alabama

M. I. Spaulding, Tuskegee Institute, Tuskegee.

Arizona

W. P. Martin, University of Arizona, Tucson.

California

H. A. Barker, University of California, Berkeley.

Meridian R. Greene, University of California, Los Angeles.

Claude E. ZoBell, Scripps Institution, La Jolla.

Colorado

H. W. Reuszer, Colorado Agricultural Experiment Station, Fort Collins.

Connecticut

Herbert A. Lunt, Connecticut Agricultural Experiment Station, New Haven.

M. F. Morgan, Connecticut Agricultural Experiment Station, New Haven.

Delaware

Henry C. Harris, University of Delaware, Newark.

District of Columbia

F. E. Allison, Bureau of Agricultural Chemistry and Engineering, U. S. D. A., Washington.

Robert H. Engle, National Fertilizer Association, Washington.

S. D. Gray, American Potash Institute, Washington.

Lewis T. Leonard, U. S. Department of Agriculture, Washington.

C. A. Ludwig, Bureau of Agricultural Chemistry and Engineering, U. S. D. A., Washington.

Oswald Schreiner, Bureau of Plant Industry, U. S. D. A., Washington.

Nathan R. Smith, Bureau of Plant Industry, U. S. D. A., Washington.

Charles Thom, Bureau of Plant Industry, U. S. D. A., Washington.

Florida

F. B. Smith, University of Florida, Gainesville.

Hawaii

Ethel K. Allen (Mrs. O. N.), University of Hawaii, Honolulu.

O. N. Allen, University of Hawaii, Honolulu.

Idaho

William V. Halversen, University of Idaho, Moscow.

Illinois

Adolph A. Hendrickson, The Albert Dickinson Co., Chicago.

Iowa

R. E. Buchanan, Iowa State College, Ames.

A. G. Norman, Iowa State College, Ames.

Kansas

Francis E. Clark, Bureau of Plant Industry, U. S. D. A., Manhattan.

Louisiana

C. S. McCleskey, Louisiana State University, University.

J. Fielding Reed, Louisiana State University, University.

Harold A. Wilson, Southwestern Louisiana Institute, Lafayette.

Maryland

Howard L. Bodily, University of Maryland, College Park.

A. G. McCall, Soil Conservation Service, U. S. D. A., College Park.

R. P. Thomas, University of Maryland, College Park.

Massachusetts

W. G. Colby, Massachusetts State College, Amherst.

Kenneth V. Thimann, Harvard Biological Laboratory, Cambridge.

Michigan

K. L. Jones, University of Michigan, Ann Arbor.

Minnesota

C. E. Skinner, University of Minnesota, Minneapolis.

Missouri

Wm. A. Albrecht, University of Missouri, Columbia.

New Jersey

D. Q. Anderson, N. J. Agricultural Experiment Station, New Brunswick.

J. A. Anderson, Rutgers University, New Brunswick.

L. S. Archibald, Rutgers University, New Brunswick.

A. W. Blair, New Jersey Agricultural Experiment Station, New Brunswick.

- Edgar A. Butters, New Jersey Agricultural Experiment Station, New Brunswick.
- Theone C. Cordon, New Jersey Agricultural Experiment Station, New Brunswick.
- Samson R. Dutky, New Jersey Agricultural Experiment Station, New Brunswick, and U. S. D. A., Moorestown.
- Jessie G. Fiske, New Jersey Agricultural Experiment Station, New Brunswick.
- Jackson W. Foster, New Jersey Agricultural Experiment Station, New Brunswick.
- Donald M. Goss, New Jersey Agricultural Experiment Station, New Brunswick.
- Louise Harrop, Rutgers University, New Brunswick.
- John G. Havens, New Brunswick High School, New Brunswick.
- Jackson B. Hester, Campbell Soup Co., Riverton.
- Werner Husmann, New Jersey Agricultural Experiment Station, New Brunswick.
- Jacob S. Joffe, New Jersey Agricultural Experiment Station, New Brunswick.
- Edward O. Karow, New Jersey Agricultural Experiment Station, New Brunswick.
- Harry Katznelson, New Jersey Agricultural Experiment Station, New Brunswick.
- Mrs. H. B. Kitchen, New Jersey Agricultural Experiment Station, New Brunswick.
- H. C. Knoblauch, Soil Conservation Service, New Brunswick.
- Joseph Lerner, Rutgers University, New Brunswick.
- H. C. Lint, Soil Conservation Service, U. S. D. A., Moorestown.
- J. F. Marrero, Soil Conservation Service, U. S. D. A., Moorestown.
- James P. Martin, New Jersey Agricultural Experiment Station, New Brunswick.
- Sigurd W. Melsted, New Jersey Agricultural Experiment Station, New Brunswick.
- Grace L. Moffatt, Earp Laboratories, Bloomfield.
- T. J. Murray, Rutgers University, New Brunswick.
- Walter M. Peacock, Deerfield Packing Corp., Bridgeton.
- F. D. Richardson, Princeton University, Princeton.
- Alfred Schenkman, Rutgers University, New Brunswick.
- H. B. Sprague, New Jersey Agricultural Experiment Station, New Brunswick.
- Robert L. Starkey, New Jersey Agricultural Experiment Station, New Brunswick.
- Jacob L. Stokes, New Jersey Agricultural Experiment Station, New Brunswick.
- Earp Thomas, Earp Laboratories, Bloomfield.

S. J. Toth, New Jersey Agricultural Experiment Station, New Brunswick.
 Selman A. Waksman, New Jersey Agricultural Experiment Station, New Brunswick.
 Benjamin Wolf, New Jersey Agricultural Experiment Station, New Brunswick.
 Boyd Woodruff, New Jersey Agricultural Experiment Station, New Brunswick.

New York

Richard Bradfield, Cornell University, Ithaca.
 Dean Burk, Cornell University Medical School, New York.
 H. J. Conn, New York Agricultural Experiment Station, Geneva.
 Alvin W. Hofer, New York Agricultural Experiment Station, Geneva.
 Conrad C. Johnson, Innis, Speiden & Co., New York.
 J. K. Wilson, Cornell University, Ithaca.

Ohio

Harold W. Batchelor, Ohio Agricultural Experiment Station, Wooster.

Pennsylvania

I. J. Hutchings, Jacob Mushroom Co., West Chester.
 James P. Sell, Lehigh University, Bethlehem.

South Carolina

T. C. Peele, Soil Conservation Service, Clemson.

Utah

Thomas L. Martin, Brigham Young University, Provo.
 D. W. Thorne, Utah State Agricultural College, Logan.

Virginia

J. L. Lockett, Virginia State College, Petersburg.
 F. S. Orcutt, Virginia Polytechnic Institute, Blacksburg.

Washington

S. C. Vandecaveye, State College of Washington, Pullman.

Wisconsin

I. H. Baldwin, University of Wisconsin, Madison.
 Robert H. Burris, University of Wisconsin, Madison.
 Lewis W. Erdman, The Nitragin Co., Milwaukee.
 Wm. C. Frazier, University of Wisconsin, Madison.
 E. B. Fred, University of Wisconsin, Madison.

W. B. Sarles, University of Wisconsin, Madison.
Robert M. Stern, University of Wisconsin, Madison.
R. K. Tam, University of Wisconsin, Madison.
Emil Truog, University of Wisconsin, Madison.
W. W. Umbreit, University of Wisconsin, Madison.
Orville Wyss, University of Wisconsin, Madison.



BRITISH DELEGATION

Left to right: Dr. A. W. Peirce, Dr. H. G. Thornton, Dr. W. H. Martin
(Director N. J. Agricultural Experiment Station), Dr. A. G. Lochhead,
Dr. T. Gibson.



Photos by T. D. Mulhern

LUNCHEON HOUR

Left to right: Prof. Jan Smit, Dr. Richard Bradfield, Dr. M. F. Morgan.



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REPORT OF MEETINGS OF COMMISSION III

WEDNESDAY, AUGUST 30, 1:30 P.M.

OPENING OF MEETINGS

SELMAN A. WAKSMAN

IT GIVES me a feeling of profound satisfaction to be privileged to open today the sessions of the Third Commission of the International Society of Soil Science. This is the seventh meeting of the Commission which I have been able to attend; I believe these were all the official meetings of this Commission since that memorable Fourth Agropedological Conference which was held in Rome, in 1924. Today we are gathered under heavy war clouds which have been overshadowing the whole of Europe during the last weeks. Many of our colleagues from various countries who were originally planning to be with us and participate in our deliberations are not here today. However, there is a marked distinction between this gathering and that scheduled just a quarter of a century ago, namely, the Third Agropedological Conference, which was to be held in Russia in 1914. That Conference was canceled because of war threats. We, on the other hand, are able to carry on and thus justify the hope of all our colleagues throughout the world that science the world over has made some progress, that science knows no political boundaries.

We all regret further that the one man who has been most active in the growth and development of the International Society of Soil Science as a whole and of the Third Commission in particular, namely, Dr. Jacob G. Lipman, former Director of this Station, is not here with us today to welcome us to this institution and to participate in our discussions. It was he who largely planned for this gathering, and it was through his efforts that the publication of the Proceedings of this meeting was made possible. I feel highly honored to be able to introduce to you Dr. W. H. Martin, the successor of Dr. Lipman as Director of the New Jersey Agricultural Experiment Station and as Dean of the College of Agriculture, who will say a few words of welcome.

ADDRESS OF WELCOME

DR. W. H. MARTIN

Dean of College of Agriculture, Rutgers University

and

*Director of New Jersey Agricultural Experiment Station,
New Brunswick, N. J.*

It is my pleasant duty to welcome you to Rutgers University and the New Jersey Agricultural Experiment Station. I regret that Dr. Lipman is not here to welcome you. It is unnecessary for me to say that we miss him. Dr. Lipman built well. Under his able guidance, the New Jersey Agricultural Experiment Station became one of the leading institutions of its kind in the United States. You all know what he did in the field of soil science. Naturally, we all respected him for his outstanding ability as an administrator and as a scientist. But more than that, those of us who were associated with him for many years learned to love him for his friendly nature and his ever-ready willingness to help the other fellow.

In the published transactions of this meeting, the Acting President of the International Society of Soil Science reports that Dr. Lipman presided at the first meeting of the Society. Rather than take the opportunity to make a formal speech, he turned to Dr. Hissink and said, "The meeting is opened; please, Dr. Hissink, what is the first point on the agenda?" This reminded me of the time that Dr. Lipman presided at the meeting of the Association of Land Grant Colleges at Houston, Texas. He appeared on the platform with a rather voluminous manuscript, which we all expected he would read. Rather than that, he stated, "It is customary that the President present a formal address. Thar she be." Then he cast the manuscript aside and drew on his wide experience to discuss informally some of the things he had in his mind.

I certainly cannot improve on his method of opening the first meeting of the International Society of Soil Science. I merely wish to say that we are most happy to have you here. You have an interesting and valuable program ahead of you, so that I am sure that your visit will be a profitable one. I hope too that yours will be a pleasant visit. We are very anxious that you make yourselves at home. If there is anything we can do for you, please do not hesitate to call on us. I can speak for all the members of our staff when I say that they are ready to serve you in every way possible.

It is now my pleasure to call upon Dr. Thornton, your president, and turn the meeting over to him—Dr. Thornton.

Dr. Thornton acknowledged the words of welcome and took over the chair of presiding officer of the Commission.

Chairman: H. G. Thornton

Secretary: I. L. Baldwin

REPORTS ON LEGUMES AND LEGUME BACTERIA

H. G. Thornton: A Suggested Explanation of the "Inefficiency" of Certain Strains of Rhizobia.

The papers in full have been published in Volume A of the Transactions of the Third Commission and are to be published in abstract form in the Proceedings of the Soil Science Society of America, Volume 4.

or from plants with efficient nodules. The juice from plants bearing efficient nodules gave a better growth than uninoculated root juice in most cases, but this difference was barely significant.

Thorne: In reference to the point which has arisen as to whether expressed juices of leguminous plants are toxic to any of the legume bacteria, I have carried on some experiments on this topic. The results obtained have been published in the *Journal of Bacteriology*. In general it was found that freshly expressed juices of some legumes exerted toxic effects on several of the strains of *Rhizobium* studied. The bactericidal effects were noted in juices expressed from frozen plants and sterilized by filtering through Pasteur-Chamberland filters.

Wilson (J. K.): Expressed juice from various legumes including tops and roots separately frozen and not frozen show that the juice from *Melilotus alba*, either tops or roots, was the only toxic juice. This was attributed to the action of the coumarin from *M. alba*.

Umbreit: I note that in your table 2 (volume A, page 23) of the abstract you report development on agar media. What is the nature of the media? Did they contain yeast extract? Did they contain a nitrogen source?

Dufrenoy: It is possible that restricted growth of nodules on legumes inoculated by inefficient strains is due to the fact that carbohydrates, instead

ADDRESS OF WELCOME

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Dean of College of Agriculture, Rutgers University

and

*Director of New Jersey Agricultural Experiment Station,
New Brunswick, N. J.*

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REPORTS ON LEGUMES AND LEGUME BACTERIA

H. G. Thornton: A Suggested Explanation of the "Inefficiency" of Certain Strains of Rhizobia.

Discussion

Waksman: Dr. Sherman, of Cornell, at one time carried out studies on the bactericidal effect of plant juices upon bacteria. Has any work been done along this line with juices of leguminous plants and their effect upon root-nodule bacteria, and what bearing does this have upon the behavior of the bacteria in the nodule?

Thornton: We have just completed statistical analysis of six experiments on the growth of nodule bacteria in media containing root juices from variously inoculated peas and soy beans. Taking these as a whole there is a very significant decrease in bacterial growth produced by the juices from plants bearing inefficient nodules as compared with juice from uninoculated plants or from plants with efficient nodules. The juice from plants bearing efficient nodules gave a better growth than uninoculated root juice in most cases, but this difference was barely significant.

Thorne: In reference to the point which has arisen as to whether expressed juices of leguminous plants are toxic to any of the legume bacteria, I have carried on some experiments on this topic. The results obtained have been published in the *Journal of Bacteriology*. In general it was found that freshly expressed juices of some legumes exerted toxic effects on several of the strains of *Rhizobium* studied. The bactericidal effects were noted in juices expressed from frozen plants and sterilized by filtering through Pasteur-Chamberland filters.

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Umbreit: I note that in your table 2 (volume A, page 23) of the abstract you report development on agar media. What is the nature of the media? Did they contain yeast extract? Did they contain a nitrogen source?

Dufrenoy: It is possible that restricted growth of nodules on legumes inoculated by inefficient strains is due to the fact that carbohydrates, instead

of remaining soluble and available to the bacteria, are stored in the cells of the meristematic cap, which cease to grow and cease thereby to be meristematic. In fact, inhibition of nodule growth on clover in fields containing soluble mineral nitrogen seems to be correlated with the storing of starch grains in the cells of the meristematic cap.

Smit: Do the poor growth and the quick decay of the nodules of ineffective strains have anything to do with bacteriophage?

Thornton: There are three reasons that make me believe that the phenomena of inefficiency in nodules are not connected with bacteriophage. *Firstly*, the early growth and cytology of the nodule before disintegration sets in, is normal in appearance though smaller in amount than is the case in efficient nodules, while the phenomena of disintegration exactly resemble those seen in efficient nodules though occurring earlier in time. *Secondly*, the typical phenomena of inefficiency appear in cultures in which an externally sterilised seed has been grown in sterile agar inoculated with a culture of the inefficient strain that shows no sign of bacteriophage. It is difficult to see how the bacteriophage could enter this system unless one makes the improbable assumption that it exists in a latent form in the laboratory culture used to inoculate the tube and only becomes active in the nodule. *Thirdly*, when inefficient nodules grown in sterilised sand are crushed and the juice filtered and added to cultures of the nodule bacteria either of the same or of different strains, there is no sign of lytic action.

Kenneth V. Thimann: The Physiology of Nodule Formation.

Discussion

Norman: Is it reasonable to conclude that the apparent lag in production of auxin by rhizobia in artificial culture is due to the fact that the auxin source might be autolyzed cell protein and not material directly formed or synthesized from any nitrogen compound in the medium? Cell counts might throw some light on this problem.

Thimann: It is possible that the auxin is produced only during autolysis, but I think that this is not the case, because auxin production, in fact, begins almost immediately, increased amounts being detectable within four days. Even at the time of very high production there are no obvious signs of autolysis, although this, of course, is only casual observation.

Thornton: H. K. Chen working at Rothamsted obtained evidence of the production of growth substance by clover nodule bacteria only where these were grown in the presence of tryptophane. Dr. Thimann's results suggest that this was due to the fact that Chen's cultures were only some 5 days old whereas in the absence of tryptophane there is a lag period before the bacteria produce growth substance, possibly due to the production by the bacteria of





Photos by T. D. Mulhern and R. H. Burris

Upper. SUPPER AT THE LOG CABIN. Left to right: Dr. A. W. Peirce, Dr. Willem Rudolfs, Dr. E. B. Fred, Mrs. H. W. Batchelor, Dr. H. W. Batchelor, Prof. Jan Smit.

Lower left. BETWEEN THE SESSIONS. Left to right: Dr. M. I. Timonin, Dr. C. A. Ludwig, Dr. J. Dufrenoy, Dr. F. E. Clark.

Lower right. WAS IT SOIL SCIENCE? Dr. H. G. Thornton, left; Dr. O. N. Allen, right.

an intermediate product. The difference in the nature of the growth substance produced by the bacteria *in vitro* and in the nodule may indicate that the first substance is active in stimulating the growth and curling of the root-hairs and the latter in causing growth of the nodule.

Artturi I. Virtanen: Mechanism of Symbiotic Nitrogen Fixation by Leguminous Plants (summarized by Dr. Sarles).

W. W. Umbreit and P. W. Wilson: Studies on the Mechanism of Symbiotic Nitrogen Fixation.

Franklin E. Allison and Sam R. Hoover: Respiration Rates of Rhizobium; Their Estimation and Significance.

Discussion of Preceding Three Reports

Burris: In general the studies carried on at Wisconsin by Dr. Wilson, Dr. Thorne, Dr. Tam, and myself are in good agreement with the results presented by Dr. Allison.

Clifton has well established the occurrence and significance of the phenomenon of assimilation by so-called resting cells. It is of course necessary to recognize this rather unfortunate limitation in our interpretation of results with resting cells.

In view of the data presented today some of our observations may be of interest. If a heavy suspension of *Rh. trifolii* 205 is placed in a Warburg flask, with glucose, and 100 micrograms of arginine or histidine is added from the side arm we find an 80 per cent stimulation of respiration which is apparent within 4 minutes. It is difficult to conceive of an 80 per cent *growth* stimulation in this short period, but assimilation or respiration may, of course, be directly stimulated. If we add 100 micrograms of the most suitable substrate for these organisms, namely succinate, the stimulation of the glucose respiration amounts to but 10 to 15 per cent.

Allison reports active cell material per unit dry weight (cells plus gum). We have adopted the nitrogen content of the rhizobia suspensions as a criterion for comparison of different suspensions, and we feel that it offers advantages over dry weight.

Thorne: Drs. Allison and Hoover have raised an interesting point with regard to the normality of organisms grown on various types of laboratory media. We believe that this question is of fundamental importance, since obviously it is desirable to study the metabolism of cells which are similar to those growing under natural conditions in the nodule. Beijerinck devised a medium which he believed would resemble the natural growing conditions of the organism. It consisted of an extract of peas plus 0.5 per cent glucose and asparagin. Virtanen has published the nitrogen content of organisms growing on this medium as ranging from 10 to 12 per cent. Obviously, it is

not possible to say whether such cells are more nearly normal than those grown on other laboratory media, for example, on media high in carbohydrate and containing ammonia salts as a source of nitrogen. For this reason it is suggested that suspensions of organisms taken directly from the nodule might be preferable, for a study of certain aspects of the bacterial metabolism, to those grown on artificial media.

A suitable technique for the preparation of such suspensions from the nodules of peas, vetch, soybeans, and cowpeas has been developed by Mr. Burris and myself; the details of the technique and examples of use of the suspensions will appear in a forthcoming issue of the *Journal of Bacteriology*.

Another point which should be mentioned is that glucose is not the most suitable substrate for respiration of the rhizobia. Succinate, for example, is respired three to six times more rapidly than is glucose by suspensions of the soybean and cowpea organisms, either from the nodule or when grown on laboratory media. The clover and pea strains respire succinate about 15 times as rapidly as glucose. In comparing the relative rates of respiration of these organisms with that of other bacteria, it appears that suitability of different substrates should be considered rather than some arbitrary standard like glucose.

Wilson (P. W.) and Wyss: Since Prof. Virtanen has questioned the validity of our criticism of his evidence for the role of hydroxylamine in the chemical mechanism of symbiotic nitrogen fixation as well as some of our results, we appreciate this opportunity to comment on his most interesting and stimulating paper. Before discussing the actual data, we wish to correct a few misapprehensions which apparently have arisen in Prof. Virtanen's mind. It is well known that numerous mechanisms have been proposed for biological nitrogen fixation, involving various types of fixed nitrogen, including NO_3 , NO_2 , NH_3 , CN , and various organic nitrogen compounds. Such mechanisms have been based almost entirely on the reported isolation of the particular compound postulated. The paragraph quoted by Virtanen refers to such studies and was not intended to apply to his researches which constitute an outstanding example of the type of investigation necessary for the establishment of a proposed mechanism, *viz.*, demonstration that the manner of occurrence of a particular compound depends on the functioning of the nitrogen fixation system. That we do not always agree with Virtanen respecting the completeness of the evidence does not imply that we either rejected the hypothesis or were critical of the manner in which the supporting data were obtained.

Exception is likewise taken to the statement that, "Wilson seems to have little confidence in the methods of organic chemistry in the elucidation of biological nitrogen fixation." The fact that we have applied the methods of organic chemistry, necessarily in a somewhat different form from that used

by Virtanen and his associates, in our researches of the past decade should be sufficient answer to this statement.^{5, 7} In addition, we wish to emphasize, as was done in the review¹⁰ and as was reiterated by Dr. Umbreit in his paper, that the term *mechanism* implies much more than mere enumeration of the chemical steps of the process. Knowledge of the physiology of the organism, of the actual agent of fixation, and especially of the characteristics of the enzyme system responsible for the fixation reaction are other aspects of the mechanism which must be comprehended before effective control of the process in nature can be attained. Whereas the methods of organic chemistry are undeniably of great value for elucidation of the chemical steps, other approaches are not only desirable but essential for solution of the more comprehensive problem. Our interests have been primarily focussed on the properties of the responsible enzyme systems; we believe that the physical-chemical approach is a more promising one for this phase of the mechanism than is that of organic chemistry. Especially to be avoided is the conclusion that these two outlooks are mutually exclusive; they are not. On the contrary, they are complementary, and both are necessary for final solution of the unsolved problems in this field.

EXAMINATION OF VIRTANEN'S EVIDENCE FOR HYDROXYLAMINE AS AN INTERMEDIATE

We now turn to examination of the lines of evidence which Virtanen has supplied in support of his hypothesis that fixation proceeds through NH_2OH via oximosuccinic acid to aspartic acid. There is no need to go into the question as to whether the excreted aspartic acid *must* represent a product of a synthetic reaction or could arise from a decomposition, since the arguments pro and con are available in the published papers. Moreover, this aspect is really secondary to the fundamental question involved: *What are the chemical steps by which molecular nitrogen is converted into aspartic acid?* Some confusion has arisen in this connection through our misunderstanding of the sense in which Prof. Virtanen used the words, "primary amino acid." Our interpretation was that he meant by this expression, the *first* amino acid formed. In this paper he evidently uses it to mean the key amino acid of protein metabolism of plants. With this usage we are in complete agreement. From the time of Schulze's experiments in the 1870's the key position of asparagine, the half-amide of aspartic acid, in the nitrogen metabolism of green plants has been generally accepted. But to recognize aspartic acid as the "primary" amino acid in this sense does not at all imply that it is the *initial* amino acid formed. The aspartic acid hypothesis of protein metabolism asserts not only that aspartic acid is used as a source of nitrogen for formation of other amino acids but also that any source of nitrogen furnished the plant,

be it inorganic or organic, will eventually appear as aspartic acid. For this reason aspartic acid does not imply any particular precursor. It could come from NH_2OH as suggested by Virtanen or from NH_3 as proposed by Winoogradsky or from organic compounds such as urea or amino acids. Any one of these sources of nitrogen may react with oxalacetic acid to form aspartic acid either directly or after liberation of the nitrogen as NH_3 .

Thus, the evidence up to the present in support of the hydroxylamine hypothesis is not critical for this compound. About all that can be safely concluded from it is that the nitrogen metabolism of legumes fixing nitrogen is very similar to the nitrogen metabolism of these same plants when supplied with combined forms. It is of interest that we arrived at the same conclusion by experiments with soybeans in which a quite different organic chemical approach was used.⁵ Under certain conditions, nitrogen is fixed in the nodule where it accumulates to such an extent that part of it is excreted as aspartic acid, in keeping with the role that aspartic acid plays as the keystone amino acid of nitrogen metabolism in legumes. Excretion appears to be associated with the presence of nodules rather than with the fact of nitrogen fixation, since these organs provide a locale in the roots for the accumulation of a high concentration of soluble nitrogen. It may be no coincidence that excretion does not occur with plants given $\text{NO}_3\text{-N}$ or $\text{NH}_3\text{-N}$, since many authorities believe that synthesis of amino acids from these compounds takes place in the leaves. If such is the case, combined nitrogen furnished plants to a level where excessive soluble nitrogen accumulates would be expected to be excreted from the leaves rather than the roots. The excretion of glutamine by grasses supplied with excessive doses of $(\text{NH}_4)_2\text{SO}_4$ apparently is an example of this. On the basis of these evidences our conclusion¹⁰ that, "in spite of the impressive and ingenious nature of the data, a critical examination reveals that they are far from being conclusive," appears to be quite valid.

During recent months, and especially in this paper, Virtanen has provided some new evidence which is decidedly more critical for his particular scheme and which undoubtedly gives it a great probability. Two points are especially impressive: first, an oxime comprising 1 to 2 per cent of the excreted nitrogen has been isolated and identified as *oximosuccinic* acid; second, an oxime has been detected, although not yet isolated, from excised nodules fixing nitrogen.

Since oximosuccinic acid is one of the postulated intermediates in Virtanen's mechanism, and since it definitely associates NH_2OH with the fixation process, its occurrence in the excreted products constitutes an example of a much more convincing excretory product than is aspartic acid. Although the same argument might be applied here with respect to its significance as has been used against finding oxime in cultures of *Azotobacter*, viz., that it is a by-product of growth, there is no evidence for the correctness of this view in the symbiotic nitrogen fixation system.

Two other lines of supporting evidence which are suggested by Virtanen in this paper also should be mentioned. These follow:

1. *Ammonia is discarded as a possible precursor of the excreted aspartic acid since glutamine (or glutamic acid) should also be formed and excreted if ammonia were an intermediate.* This suggestion should lead to some very interesting research in plant biochemistry, as it implies that amide metabolism in plants assimilating ammonia is different from that of plants assimilating nitrates or molecular nitrogen.

2. *The assimilation of nitrate-nitrogen proceeds via hydroxylamine rather than the traditional ammonia.* This view, proposed by Lemoigne, has not yet been generally accepted by plant biochemists. Nightingale, commenting on Lemoigne's work, in his recent review⁴ says that "at present any discussion concerning the possible significance [of hydroxylamine] would be purely speculative." If the formation of aspartic acid from nitrate does proceed through hydroxylamine, it is surprising that red clover should do better when fixing nitrogen than when supplied with KNO_3 . It might be expected to prefer fixation to NH_3 -nitrogen but hardly to NO_3 -nitrogen.

We have tried to emphasize in this paper that the data offered by Virtanen in support of his hypothesis are of two types which should be sharply differentiated: (a) nonspecific evidences, such as the finding of aspartic acid in the excreted products, oxalacetic acid in the plant, and the fixation of nitrogen by excised nodules in the presence of oxalacetic acid; (b) specific evidences represented by the identification of oximosuccinic acid in the excreted products. The nonspecific evidences afford a necessary but not an adequate support and could be used equally well in favor of other hypotheses, e.g., that based on the formation of ammonia. In this particular case the specific evidences confirm the suggestions based on the nonspecific support. This happy outcome, however, is not always observed, and it is for this reason that experiments in support of a proposed biological mechanism must be critically analyzed in order that the nonspecific be clearly separated from the specific.

THE SIGNIFICANCE OF EXCRETION FOR MIXED CROPPING

Before concluding, we wish to add a few remarks concerning the particular aspects of excretion in which we have been primarily interested, *viz.*, the probable role it plays in the acknowledged benefits of mixed cropping. Although Prof. Virtanen and his associates in Finland have experienced no difficulty in securing excretion with several species of legumes, workers at other stations have not been so fortunate. Ludwig and Allison³ at Washington, D. C., Trumble⁶ at Waite Institute, Australia, Bond¹ at Glasgow, Scotland, and we ourselves¹¹ at Wisconsin have conducted extensive experi-

ments over a long period of years with almost uniformly negative results. By adopting rather artificial conditions for growing plants, as by shading or by use of low temperatures, we have succeeded in obtaining excretion, but positive results with the plants grown under the natural environmental conditions of our station have been few.¹² Evidently, there exists a fundamental difference between the manner in which plants develop at Helsinki and at the other experiment stations. We have been particularly interested in detection of biochemical differences in the plants which may be associated with the occurrence or nonoccurrence of excretion. At present two distinct variations between the two sets of plants may be cited, as follows:

1. *Content of oxalacetic acid.* Virtanen reports up to 500 micrograms of oxalacetic acid per gram of wet tissue in legumes grown at Helsinki, whereas we have found none in plants grown under Wisconsin conditions. In his paper and in a recent note,⁹ Virtanen decides that our failure to duplicate his results is because the methods we used will not detect oxalacetic acid in plant tissues. This conclusion is most surprising in view of the fact that we used the identical methods which were originally employed by Virtanen and his co-workers.⁸ It is curious that they could detect oxalacetic acid by these methods at one time and now declare their use to be the cause of our failure to confirm their findings. Aside from this contradiction, it is to be observed that we have already published the results of a careful study in which the Ostern manometric method, which is standard in biological laboratories for the estimation of oxalacetic acid, was investigated for use with plant tissue.¹³ Under the conditions outlined in our paper, no difficulty was experienced in quantitatively recovering oxalacetic acid when this is added at any stage in the method. Our failure to detect oxalacetic acid in plants grown at the Wisconsin station is not intended as a criticism of the evidence for the formation of oximosuccinic acid. It appears likely that oxalacetic acid would be present in most plant tissues because of its role in the respiration cycle; ordinarily, its level may be too low to be detected by the Ostern method, which has a sensitivity of approximately 10-20 micrograms per gram. Even though oxalacetic acid was present at a much lower level, its concentration would be sufficient to tie up the postulated NH_2OH , the occurrence of which in the free state is most transient. The significant fact which we wish to emphasize is the quantitative difference, *viz.*, leguminous plants grown under Finnish conditions contain a surprisingly high level of oxalacetic acid, whereas those grown at Madison, Wisconsin, do not.

2. *Fixation by excised nodules.* A second difference in the plants grown at the two stations is in the ability of their nodules, when supplied with oxalacetic acid, to fix nitrogen apart from the host plant. Evidently, no difficulty is encountered when nodules from plants grown at Helsinki are

used. We have completed about 20 experiments at our station using nodules from Finnish varieties of peas inoculated with a Finnish culture as well as nodules from native varieties of pea, soybean, and cowpea. In no case have we been able to detect any uptake of free nitrogen either by the Kjeldahl method or by a special gasometric method we have developed. Virtanen's criticism of our gasometric method evidently rests on a misunderstanding of its principle. Since we have in press² a detailed account of the theory and applications of the method, it is necessary here only to observe that in all our tests we have included *Azotobacter* controls, which demonstrated that the method would readily detect the uptake of as little as 0.5 mgm. N.

We have discussed two major biochemical differences in the plants grown in the different environments which might be correlated with the phenomenon of excretion. There are probably others which will be revealed by further research. When a number of these are uncovered, we shall probably be in a much better position to decide why excretion occurs in one locality and not in another. It is perhaps unfortunate that other workers have been unable to secure excretion to the same extent as have Prof. Virtanen and his collaborators, since confirmation of their important findings would then undoubtedly be available. In view of the extensiveness and high caliber of their experimental technique, however, we are certain that few would hesitate to accept their results even though other workers do not have the opportunity to repeat the experiments. By the same token, the negative results appear to be equally well established. The immediate problem is to discover the cause of what appears to be a marked difference in the biochemistry of the two sets of plants. We believe that resolution of the difficulty will be achieved much earlier if we accept one another's findings as being equally valid and concentrate on determining the biological reasons for the difference rather than assume that the investigator is at fault.

REFERENCES

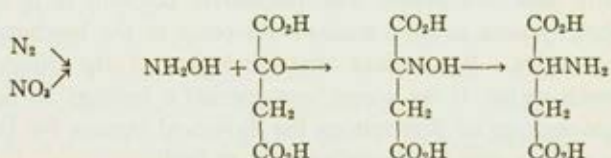
- ¹ BOND, G. 1938 *Ann. Bot. (n.s.)* 2: 61-74.
- ² HURWITZ, C., AND WILSON, P. W. 1939 *Indus. and Engin. Chem. Analyt. Ed.* 11: (in press).
- ³ LUDWIG, C. A., AND ALLISON, F. E. 1937 *Bot. Gaz.* 98: 680-695.
- ⁴ NIGHTINGALE, G. T. 1937 *Bot. Rev.* 3: 85-174.
- ⁵ ORCUTT, F. S. 1937 *Soil Sci.* 44: 203-216.
- ⁶ TRUMBLE, H. C., AND STRONG, T. H. 1937 *Aust. Advisory Council Sci. and Indus. Research Bul.* 105.
- ⁷ UMBREIT, W. W., AND BURRIS, R. H. 1938 *Soil Sci.* 45: 111-126.
- ⁸ VIRTANEN, A. I., LAINE, T., AND ROINE, P. 1938 *Suomen Kemistilehti* B 11:25.
- ⁹ VIRTANEN, A. I., AND ARIHIMO, A. A. 1939 *Nature* 144: 36.
- ¹⁰ WILSON, P. W. 1939 *Ergeb. Enzymforsch.* 8: 13-54.
- ¹¹ WILSON, P. W., AND BURTON, J. C. 1938 *Jour. Agr. Sci.* 28: 307-323.
- ¹² WILSON, P. W., AND WYSS, O. 1937 *Soil Sci. Soc. Amer. Proc.* 2: 289-297.
- ¹³ WYSS, O., BURRIS, R. H., AND WILSON, P. W. 1939 *Soc. Expt. Biol. and Med. Proc.* 40: 372-375.

Artturi I. Virtanen and A. A. Arhimo: Formation of Amino Acids in Green Plants with Nitrate as the Nitrogen Source.

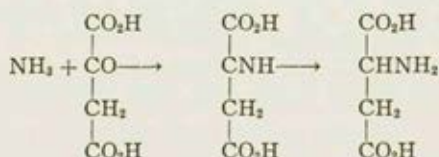
(Note forwarded to be presented in discussion. Read by Dr. Sarles.)

We have noted that when growing pea or oat is placed for 24 hours in a 2 or 4 per cent KNO_3 solution—the roots immersed, the stems above liquid—nitrite-N and hydroxylamine can be clearly demonstrated in the roots. The same result was obtained both in the light and in the dark. Hydroxylamine was determined by oxidizing it with iodine to nitrite, which was then determined.¹ If the plants are kept in ammonium sulfate solution, hydroxylamine is not formed or its formation is too weak to be shown with certainty.

These observations support the theory earlier advanced by one of us² that when nitrate acts as the nitrogen source for plants, hydroxylamine is formed as a reduction product. NH_2OH then reacts with oxaloacetic acid and probably with ketoglutaric acid, forming the corresponding oximes, from which aspartic and glutamic acids are formed through reduction. The presence of oxaloacetic acid and ketoglutaric acid in the green plants has been noted by us. As the hydroxylamine reacts readily even in very dilute solutions especially with oxaloacetic acid, the formation of oxime is a logical result of the formation of hydroxylamine in the plants. Only in case no keto acids were present, could hydroxylamine be reduced to ammonium. The formation of amino acids in plants growing on nitrate-N would thus take place in the same way as in the fixation of atmospheric nitrogen, thus:



When ammonium salts form the nitrogen source of plants, ammonium evidently reacts, without oxidation to hydroxylamine, with oxaloacetic or ketoglutaric acid, while aspartic acid or glutamic acid dehydrase acts as a catalyst. The reaction then is known to occur in the following way:



¹ BLOM, *Ber. Deut. Chem. Gesell.* 59: 121 (1926).

² VIRTANEN, *Cattle Fodder and Human Nutrition with Special Reference to Biological Nitrogen Fixation*. Cambridge (1938); *Virtanen and Laine, Biochem. Jour.* 33: 412 (1939); *Virtanen, Svensk Kem. Tidskr.* 51: 21 (1939); *Trans. Third Comn. Internat. Soc. Soil Sci. A*, 4-19 (1939).

Although the final result is the same as when nitrate or ammonium nitrogen forms the nitrogen source of plants, the course of the reaction is different, according to the foregoing conception.

A. Demolon et A. Dunez: Sur la lyso-resistance du *B. radicola* et son importance pratique (presented by Dr. Dufrenoy).

H. Katznelson: Bacteriophage and the Legume Bacteria.

Discussion of Above Two Papers

Hofer: Is there any simple reliable way of determining the presence of phage in alfalfa-sick fields?

Katznelson: Several methods may be used to determine its presence, but they are hardly simple. 1. Demolon and Dunez examine nodules from plants on "fatigued" soil, and if these show granulation and intense cellular degeneration they consider that phage is responsible. 2. Pieces of nodules, which are first sterilized and carefully washed, are placed on streaks of susceptible alfalfa strains, and development of lytic areas is observed. These observations must be confirmed, however, by isolating the lytic principle and testing it for "multiplication" by serial transfers and plaque formation. 3. Perhaps the most reliable method is to incubate a composite sample of soil from the immediate vicinity of the plant roots with a yeast-water medium for several days, filter through paper and Berkefeld, and test the Berkefeld filtrate against susceptible strains.

J. K. Wilson: Symbiotic Promiscuity in the Leguminosae.

Discussion

Albrecht: Was symbiotic promiscuity more general with legumes more commonly grown in the Southern United States or with those on soils of lower fertility?

The increased promiscuity in agreement with increased cross pollination has an interesting possible connection with decreasing soil fertility (increasing deficiency in bases by the soil or increasing hydrogen concentration or acidity). Increased hay fever in the last 25 years caused by weeds of higher pollen production per plant suggests an agreement with declining soil fertility that supports only sparse plant populations which survive only by much pollen production per plant for cross inoculation. Thus may we not see cross pollination, symbiotic promiscuity, and declining soil fertility in terms of bases as a significant relation?

Sarles: Unpublished work carried on three years ago at the Wisconsin Agricultural Experiment Station by Mr. E. W. Ruf and myself, although conducted under different experimental conditions, failed to bear out the

conclusions drawn by Prof. Wilson. Disinfected seeds of lentils, common peas, Austrian winter peas, sweet peas, common vetch, hairy vetch, broad beans, and Cicer were planted in half-gallon pots of sterile, nitrogen-poor sand. Some seed of each kind of plant were left uninoculated; other seeds of each kind of plant were inoculated with a heavy suspension of root-nodule bacteria at the time of planting. Nineteen strains of *Rhizobium leguminosarum*, one strain of *Rhizobium* from Cicer, one strain of *Rhizobium* from cowpea, and one strain of *Rhizobium phaseoli* were used in these tests. The plants were grown under aseptic conditions (provided with sterile, modified Crone's nutrient solution and watered with sterile distilled water for 3 to 6 weeks). A new experimental set-up was made every 3 to 4 weeks over a period of 12 consecutive months.

In no case did *Rhizobium leguminosarum*, *Rhizobium phaseoli*, or the *Rhizobium* from cowpea form nodules on Cicer. In no case did *Rhizobium phaseoli*, the *Rhizobium* from cowpea, or the *Rhizobium* from Cicer form nodules on any of the pea, vetch, or broad-bean plants.

These results are presented in support of the belief that the rhizobia are not so promiscuous as Prof. Wilson suggests. The recently reported results of Carroll, Mrs. Conklin, Thorne and Walker, Raju, Reid, Allen and Allen, and Bushnell and Sarles also indicate that bacterial-plant groups are rather definite. At least, under the conditions of the experiments performed by these workers, conditions admittedly different from those employed by Prof. Wilson, considerable specificity in infectiveness has been demonstrated.

Batchelor: At the first three meetings of the Soil Science Society of America, Dr. Wilson discussed his preliminary work on the problem he has now given in some detail. To some of us his ideas appear to be too radical a departure from orthodox views on legume nodulation to permit their ready acceptance. To others of us his actual statements appear to be very conservative, considering the manifold implications of the new concepts he is progressively formulating. As part of the discussion that followed Dr. Wilson's first report at the meetings of the S. S. S. A. in Washington in 1936 I was bold enough to predict that his work would mark a new era in the study of symbiotic nitrogen fixation by legumes. I further ventured the suggestion that his new work, when completed, would go down in the history of soil biology as the most revolutionary series of contributions that have been made since the nodule-forming bacteria were discovered. I do not feel that anything I may say will either add to or detract from Dr. Wilson's prestige as a scientist, but even in the face of the skepticism evidenced today in the discussion of his paper, I am happy to repeat my earlier predictions and to affirm my belief that this work is about to usher in a renaissance in the study of symbiotic nitrogen fixation.

Fred: I should like to raise two questions:

The first concerns the unnatural conditions under which the plants were grown. Were not the plants grown in closed containers, and was there not a large number of plants in each? Still more important, did not the nutrient solution contain sucrose? These conditions are quite unlike normal field conditions.

In the second place, are the bacteria from these new crosses as described by Prof. J. K. Wilson efficient; that is, do the organisms in association with the higher plant fix nitrogen? In other words, have you been able to show that the bacteria from these unusual crosses are of benefit to their host plant?

Wilson: I do not think the abnormal conditions play an important part. As regards nitrogen fixation, I have not as yet had time to make quantitative tests.

Lewis T. Leonard: Bacteria Associated with *Gleditsia triacanthos* L.

Discussion

Thornton: It is one of the curiosities of *Gleditsia* that it belongs to the small group of leguminous plants that occurs in Cretaceous and early Tertiary strata. The absence of typical nodules on this group of plants suggests the possibility that the association of the Rhizobia with the leguminosae did not become established until after this time. The spread of nodule bearing legumes throughout the world during the Tertiary Epoch and the consequent improvement in food supply, may well have had its influence on the remarkable development of mammalian life that took place over this period.

Wilson (J. K.): Referred to positive results with *Cicer* inoculated in the greenhouse and negative in the field. He noted that this comment is already in his original paper.

Leonard: No nodules from location originally reported positive by Friesner.

Wm. A. Albrecht: Some Soil Factors in Nitrogen Fixation by Legumes.

Discussion

Umbreit: I recognize that you have been discussing these matters from a soils point of view, but from the point of view of mechanism I have two questions to ask. First: Is there evidence that any of the factors you have been discussing are specific in nature, i.e., do any of them operate differently on plants fixing nitrogen symbiotically than on plants fed combined nitrogen? Second: Do you regard the loss of nitrogen in certain cases you have noted as evidence of nitrogen excretion? Does the plant actually lose nitrogen to

the clay, what is its amount, and can you recover the lost nitrogen from the clay?

Albrecht: As soil factors, their functions may seem to be equally as specific as texture, degree of calcium saturation, or amounts of exchangeable magnesium or potassium can be said to be specific. If one should define "specificity" as its behavior independently of all other factors, then these factors are not specific. As is true in most biological activities, they are interrelated, as was shown for calcium and phosphorus. No test was made of the increase in nitrogen in the colloidal clay where the total nitrogen content in the crop at the close was less than that of the seed at the outset. But since bases moved back into the clay from the crop, as shown by increases in the pH figure, it is reasonable to believe that nitrogen moves as a positive ion the same as others.

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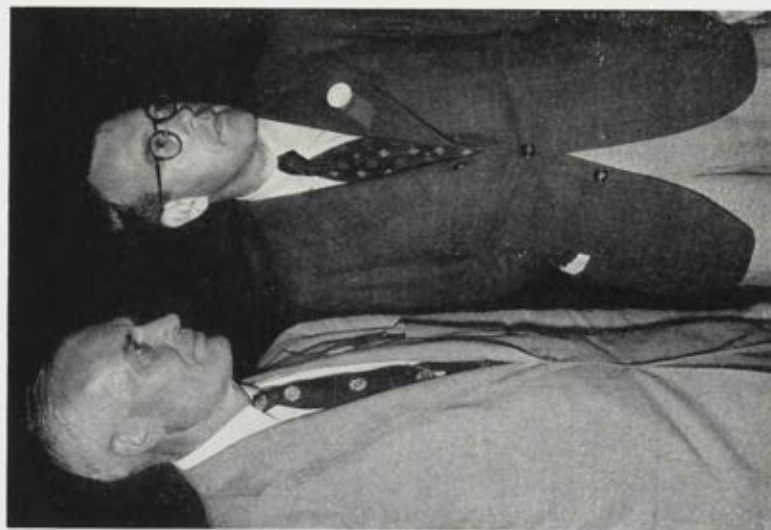


Photo by T. D. Mulhern
Dr. J. K. Wilson, left; Dr. F. Chodat, right.



Photo by T. D. Mulhern
AN EXCHANGE OF IDEAS. Dr. Charles Thom,
left; Dr. J. Duirenoy, right.

THURSDAY, AUGUST 31, 9 A.M.

Chairman: Dr. Charles Thom

Secretary: Dr. I. L. Baldwin

REPORTS ON MICROBIOLOGY OF SOIL ORGANIC MATTER

Arthur Geoffrey Norman: Observations on the Hypochlorite Oxidation of Decomposed Plant Residues.

Discussion

Waksman: This paper represents an interesting contribution to our knowledge of the process of "humification." It would be highly desirable, from a practical point of view, as in preparation of composts, especially for mushroom production, to have a method whereby one would be able to measure the extent of decomposition. The mere loss in weight or increase in ash content is not sufficient, since materials of different carbohydrate, lignin, and nitrogen contents have to be decomposed to different extents in order to bring the material down to the same degree of decomposition, or "humification value," if you please. Many methods have been proposed to measure this value, such as acid hydrolysis, oxidation value, and others. For practical purposes, the method has to be simple and rapid. A method of this nature is still needed.

Scheffer: Wir werden in Deutschland eine Reihe anderer Methoden wie z. b. die Wasserstoffsuperoxydmethode oder die Acetylbromidmethode für Bestimmung des biologisch schwer angreifbaren Ligninanteils an. Ich möchte Dr. Norman fragen, ob er die Hypochloritmethode mit diesem Verfahren vergleichen hat. Es ist anzunehmen dass die Hypochloritmethode, die auf der Oxydation des in Frage kommende Stoffe besucht, ähnliche Ergebnisse wie die H_2O_2 -methode liefert. Andererseits ist zu behalten dass der HCl-Anteil der Hypochloritmethode auch andere Stoffe als Lignin wie vor allen Kohlenhydrate in Lösung bringt.

Batchelor: Most of us are probably familiar with the large amount of excellent work on lignin chemistry that Dr. Norman has contributed. In the hope that it will make it possible for Dr. Norman to give us a more specific idea of his present methods of preparing lignins I would like him to discuss as briefly as possible his present methods of preparing lignin.

Norman: In answer to Dr. Waksman: Inasmuch as hypochlorite reacts primarily with lignin and nitrogenous groupings, which are the constituents that accumulate as decomposition proceeds, some procedure akin to the one

described might perhaps be used for the determination of degree of decomposition in the preparation of composts, though the search for such a procedure was not the main purpose of the work.

In answer to Dr. Scheffer: Oxidation by hypochlorite has not been compared with the peroxide or acetyl bromide methods, though the reaction is of an entirely different order from that brought about by the former. As commonly carried out, peroxide treatment, particularly in the presence of soil, brings about virtually complete oxidation of the residues, including carbohydrates such as cellulose. Hypochlorite oxidation in the high dilutions used in this work is almost specific with respect to lignin and amino groups and is not complete even for the lignin molecule. The reaction is somewhat more satisfactory in alkaline solution than in acid, though in the latter case oxidation proceeds further.

In answer to Dr. Batchelor: In the preparation of lignin one has the choice of extraction by alkaline reagents, which give good yields only under drastic conditions; by alcoholysis, with which yields are invariably low; or by strong acid-exclusive methods designed to remove all other constituents and leave only the lignin as an insoluble residue. Provided proper precautions are taken to prevent interference by certain other substances the last is probably the most satisfactory for decomposition studies, in that the whole of lignin is obtained. Fatty constituents and resins have to be removed by pretreatment, pentose containing polysaccharides by prehydrolysis, and the protein lowered as far as possible by any suitable extraction. The last at present provides the chief difficulty, and lignin preparations from materials of high nitrogen content are by no means free of condensed nitrogen attached to the lignin. This is not protein but protein fragments resulting from the strong acid treatments that are given to remove the polysaccharides.

Francis E. Clark and Charles Thom: Effects of Organic Amendments upon the Microflora of the Rhizosphere of Cotton and Wheat.

Thornton: I am very pleased to see that, as judged by the smoothness of the figures given, the authors have succeeded in the difficult technique of obtaining a satisfactory accuracy in estimating bacterial numbers in the root surroundings. I think the qualitative aspect should also be followed up. The effect of plant roots on the types of organisms developing and upon the competition between them is of first importance. We have found that when two strains of pea nodule bacteria are placed in contact with growing pea roots, some increased growth of the bacteria is induced by the presence of the roots, but often one strain alone increases and suppresses growth of the other strain almost completely. The factors controlling this competition require further elucidation.

Timonin: In conducting an experiment of the same nature, we obtained similar results, namely, that manurial treatment has little or no influence on the microbial accumulation in the rhizosphere of the plant. Further, I should like to ask the opinion of Dr. Clark as to the definition of "rhizosphere." Previous workers reported results obtained from the macerated roots and the soil attached to them. In our work we included only the soil attached to the roots, and the calculations of the microbial population were made per gram of soil on the basis of moisture-free soil.

Starkey: As regards the specific distinction of the rhizosphere, it would seem that little is to be gained by emphasizing that any of the usual methods of sampling of soils accurately represents the rhizosphere alone. The samples, almost without exception, represent different degrees of dilution of soil unaffected by roots with material derived from the roots. The rhizosphere can hardly be distinguished except by microscopic observations, which give a more exact picture of the very localized regions where root hairs and small and large root parts affect the microbial population. For precise distinction it would seem obligatory to refer to the particular root-soil region or parts that are concerned.

Thom: Dr. Clark's contribution here tends to emphasize that the term "rhizosphere" as originally used does not apply to the fractionation we now find necessary. The actual microflora of the root suspension has now been shown to be more or less fully independent of the surrounding earthy mass. The addition of amendments—manure, plant remains, etc.—causes tremendous changes in the total microflora of the earthy mass to be brought about without immediately involving great changes in the actual microflora of the surface of the roots themselves. It is probably desirable to drop the term "rhizosphere" for this definite fraction of the total population which is readily separable in examining the total area.

Lochhead: At Ottawa, we found, as intimated by Dr. Timonin, that in agreement with the findings described by Dr. Clark, field application of fertilizers produced comparatively little change in the rhizosphere population. However, under our conditions, fertilizer applications (including farmyard manure) on a field scale, produced relatively little differences in numbers of microorganisms in the control soil. Much greater differences in control soils were noted in the same soil supporting different crops than between soils differently fertilized growing the same crop. This would tend to confirm the opinion that the plant exerts a greater effect on the soil population than does the fertilizer treatment. In this connection, I should like to inquire whether Dr. Clark's organic amendments were employed in quantities which would be comparable to field applications.

Clark: The organic amendments employed in the greenhouse were those recommended by Dr. Fellows for the control of take-all, and the rates of

application were considerably heavier than field rates. However, some of the cotton plat fertilizations made by Mr. Hooton at Greenville were practical field applications, and we did find considerable differences in numbers of microorganisms in such plats, in comparison with the corresponding check plats. Increases were especially pronounced when moisture and temperature conditions were not limiting factors; during the spring or early summer months, increases of one hundred million or more microorganisms per gram over the check soil populations were noted. Such differences did not exist later in the summer. Your observation on the effects of different crops upon soil populations is of interest, not only from the point of view of soil sanitation, but also because of reports of differences in crop productivity, even in the absence of disease, following variations in the order in which given crops were employed in a rotation.

Selman A. Waksman: The Method of Proximate Analysis and Its Application to the Study of Plant Residues, Composts, and Humus Formations.

Discussion

Norman: While it is undeniably true that great advances have been made in the understanding of the processes of microbiological decomposition of plant materials by the application of the chemical methods forming the proximate system described by Dr. Waksman, nevertheless some caution is necessary lest we ascribe to it too great an appearance of precision in terms of actual plant constituents. There is considerable risk in the unintelligent use of such a system based on empirical fractionations. Dr. Waksman recognizes and has pointed out that certain of the fractions are neither homogeneous nor very clearly definable. For example, he is careful in this paper to refer to "dilute acid hydrolyzable constituents" and "concentrated acid hydrolyzable constituents" though elsewhere these have been grouped specifically as hemicelluloses and cellulose respectively. The dilute acid hydrolyzable fraction certainly does not accurately represent the hemicellulose fraction of plants and will be quite differently composed, for example, in decayed residues and parent materials. We must then avoid the complacent acceptance of such a scheme to the point that rigidity sets in, which might prevent the progress that would follow the use of more refined analytical methods when they are developed. The history of the use of the conventional methods of foodstuff analyses should be a warning. When developed first, they, like the proximate system, resulted in a great step forward, but later and to the present, have actually retarded and confused digestibility studies because certain of the fractions concerned are artificial and unreal. The proximate system in its own field is far more satisfactory, but its very successful use to date should not blind us to certain shortcomings.

Scheffer: Unter allen in den letzten Jahrzehnten angewandten Methoden zur Untersuchung des Humuskörpers in Böden oder organischen Düngemitteln nimmt zweifellos die "proximate analysis" in der von S. A. Waksman entwickelten Form eine ganz hervorragende Stellung ein, da sie mit chemischen Reagenzien die gesamte organische Substanz des Bodens und der Dünger analysiert und sie in einzelne biologisch trennbare Stoffgruppen zu trennen gestattet, war nicht nur für die Erforschung der Tätigkeit der Mikroorganismen des Bodens, sondern auch für die Erforschung der Reaktionserscheinungen im Boden ("Physiologie des Bodens") von grösstem Wert ist. Daher ist die "proximate analysis" mit Recht eine chemische biologisch-physiologische Methode zu nennen. Der weitere Ausbau insbesondere eine Vereinfachung mancher Arbeitsgänge wäre jedoch noch zu wünschen. Auch der zur Zeit noch vorhandene Schönheitsfehler der Analyse, höchstens nur 90 Prozent, teilweise sogar nur 80 Prozent und darunter, der analysierten organischen Substanz zu finden, ist in der Literatur bereits kritisiert worden und sollte noch abgestellt werden.

So verschiedenartig und mannigfaltig die gesamten organischen Substanzen des Bodens, die wir Humus nennen, auch aufgebaut sind, so lassen sie sich doch in folgende zwei Hauptgruppen teilen: 1. Die wasserlöslichen und ebenso die in verdünnten und auch in stärkeren Säuren löslichen und hydrolysierbaren Stoffgruppen werden von den Bodenorganismen verhältnismässig schnell angegriffen und zersetzt. Sie dienen als Nahrung zum Aufbau oder als Energiequelle: Diese Stoffgruppen habe ich daher mit "Nährhumus" bezeichnet. 2. Die in 80 Prozentiger Schwefelsäure unlöslichen Stoffe werden von den Mikroorganismen des Ackerbodens unter normalen Verhältnissen nur noch schwach angegriffen und scheiden daher als Nahrungsquelle praktisch aus. Damit verlieren sie aber keineswegs ihre Bedeutung für das Bakterienleben im Boden. Denn diese Stoffgruppe, die sich wiederum aus chemisch verschieden aufgebauten Substanzen zusammensetzen wie Lignin und ligninartige Stoffe, Humine, Huminsäuren, schwerzersetzbare Proteine usw.usw., ist der wichtigste Bestandteil des Humuskörpers aller fruchtbaren Böden. Er ist im Boden unter gewissen klimatischen Bedingungen (im Schwarzerde-, Braunerde- und Podsolklima) schwer zersetzbar und soll daher "Dauerhumus" genannt werden. In Verbindung mit Kalk wird er schwerbeweglich und reichert sich daher in dieser Form in vielen Böden an. Ein Musterbeispiel hierfür ist die Schwarzerde, in der diese Humusform den weitaus grössten Anteil des gesamten Humuskörpers ausmacht und dort an Kalk und an anorganische Sorptionsstoffe gebunden in einer wasserunlöslichen Form vorliegt. Ohne auf die Eigenschaften der beiden Humusformen Nährhumus und Dauerhumus im einzelnen einzugehen, so soll doch das Eine nochmals herausgestellt sein, dass beide Formen für das Leben im Boden, für die Fruchtbarmachung, Erhaltung und Mehrung der Fruchtbarkeit der Böden

gleich wichtige Aufgaben besitzen. Es sei noch erwähnt, dass der Dauerhumus als Sorptionsträger (Wärme, Wasser, Nährstoffe) und als Puffer-substanz für die Mikroorganismen und für die höheren Pflanzen von ganz besonderer Bedeutung ist. Die Sorptionserscheinungen, ebenso die Gesetzmässigkeiten an verschiedenen organischen Körpern sind in den letzten Jahren in vielen Arbeiten untersucht und in Vergleich mit den Erscheinungen an anorganischen Sorptionsträgern dargestellt und gewürdigt werden. Die Zusammensetzung des Dauerhumus war gleichfalls Gegenstand vieler neuerer Untersuchungen.

Die Ermittlung besonderer Humusformen in den verschiedenen Bodentypen (Springer), die Untersuchungen über Humustonkomplexe (L. Meyer), die Anwendung neuer Extraktionsmethoden, die u.a. zur Feststellung kolorimetrischer Eigenschaften der Humusstoffe, insbesondere des Dauerhumus wie Farbtiefe, Farbton (Simon, Springer, Hock, Scheffer) verwandt wurden, haben wertvolle Ergebnisse gebracht, worauf hier nur hingewiesen werden kann. Die Einführung der Acetylbromidmethode gestattet nach Springer die Bestimmung echter Humusstoffe, die wir jedoch richtiger als "echte Dauerhumusstoffe" bezeichnen wollen. Während die Schwefelsäuremethode (80 prozentiger) im Rückstand eine Reihe verschiedener Stoffgruppen, die ihrem biologischen und chemisch-physikalischen Verhalten nach den "echten Dauerhumusstoffen" sehr stark ähneln, abtrennt, umfasst sie zugleich eine Reihe von Verbindungen wie z. B. die Lignine oder ligninähnlicher Verbindungen, die noch umwandlungsfähig sind und erst allmählich in echte Dauerhumusstoffe übergehen. Beide Verfahren, die Schwefelsäuremethode wie auch die Acetylbromidmethode haben daher ihre besondere Bedeutung und charakterisieren den Dauerhumus recht gut. Es ist wohl selbstverständlich, dass die N-haltigen Komponenten in allen Fällen zu ermitteln sind.

Die hier nur angedeuteten Verfahren dienen neben anderen Untersuchungen zur Beurteilung nicht nur der Humusformen der Böden, sondern auch der organischen Düngemittel. Die Frage der Mehrung und Steigerung der Fruchtbarkeit der Böden hat für viele, ja für fast alle Länder eine fundamentale Bedeutung. Dabei verstehe ich unter Mehrung der Fruchtbarkeit nicht die Steigerung der Ernten durch Anwendung erhöhter Mengen Handelsdüngemittel, sondern zunächst die Verbesserung der physikalischen, chemischen, und biologischen Eigenschaften des Bodens mit Hilfe brauchbarer bodenverwandter Stoffe. Die Lehre von der Bildung und Entstehung der fruchtbaren Böden, die die wissenschaftliche Bodenkunde ausgebaut hat, gibt der Humusforschung die Arbeitsrichtung. Die Untersuchungen der Schwarzerden zeigen, dass eine Anreicherung des Humus unter bestimmten klimatischen Bedingungen möglich ist. Der Schwarzerdehumus besteht zu über 70 Prozent aus echtem Dauerhumus, der an Kalk und anorganische Tonteilchen gebunden ist. Er besteht nur zu einem kleinen Teil aus Nähr-

humus. Allerdings wird den Mikroorganismen in diesen Böden in Form von Ernte- und Wurzelrückständen genügende Nahrung geliefert. Wie eigne Untersuchungen ergaben, ist der Dauerhumus dieser Schwarzerdeböden sehr stabil. Als Beispiel führe ich an, dass der Humusgehalt der ungedüngten Böden des sog. "Ewigen Roggenbaus Halle-S." im Laufe einer 50 jährigen Versuchsdauer unverändert geblieben ist. Die Düngung mit "Dauerhumus"—reichen Düngemitteln und ihre Gewinnung im landwirtschaftlichen Betrieb in Form von gut verrottetem Stallmist oder noch besser als Kompost (Humus + Kalk + Boden) verdienen in Zukunft besondere Beachtung und bilden auch zur Zeit mehrfach Gegenstand eingehender Untersuchungen.—Jedes Humusdüngemittel enthält wichtige Pflanzennährstoffe und Humusstoffe. Die Bewertung dieser Düngemittel sollte in erster Linie nach ihrem Gehalt an Humusstoffen, und zwar an solchen Humusstoffen, die den Boden auf die Dauer in physikalischer, chemischer, und biologischer Hinsicht günstig beeinflussen, d. h. fruchtbarer machen erfolgen. Die beiden Humusformen Nährhumus und Dauerhumus haben ihre besonderen Aufgaben, die sie für Boden, Mikroorganismen, und Pflanze am besten erfüllen, wenn sie in einem harmonischen Verhältnis zu einander und mit den notwendigen Pflanzennährstoffen stehen. Meine Aufgabe war es, die Aufmerksamkeit auf den für den Aufbau des Bodens so überaus wichtigen Humus-anteil, den Dauerhumus, zu lenken.

Noch ein Wert zum C/N verhältnis. Das C/N verhältnis wird oft zur Charakterisierung organischer Stoffe verwandt und zwar, wie die Literatur zeigt, nicht immer mit Erfolg. Diese Grösse hat nur dann biologischen Wert, wenn die Zersetzbarkeit der C- und der N- komponente gleichmässig gegeben ist, wie es z.B. bei leichtzersetzbarem Pflanzenmaterial der Fall ist. Besteht aber die C- komponente aus schwarzzersetzbaren Stoffen, oder aus Dauerhumus, so sagt das C/N verhältnis nichts mehr über biologische Umsetzungen wie CO₂-bildung; oder Nitrifikation aus. Das "biologische" C/N verhältnis sollte daher nur aus den umsetzbaren C-, bzw. N- mengen gebildet werden.

Waksman: The missing 20-25 per cent of the humus constituents are due to compounds, largely polyuronides, which do not give 100 per cent sugar on hydrolysis, but much lower amounts. In the use of the proximate method of analysis, we purposely tried to avoid misleading terms, such as "true humus," in spite of the fact that the acetyl-bromide method may frequently give valuable information concerning the nature of certain humus constituents.

Joffe: The pedologists do not claim that the "Dauerhumus" of the chernozem is due exclusively to formation of Ca-humates. It is primarily due to the colloidal properties of the humus. The heat of the summers in the chernozem belt and the frosts of the winters coagulate the humus to an irreversible state. Wiegner has shown this to be true. If Dr. Scheffer would

bring his "Dauerhumus" of the chernozem to the zone of laterites it would decompose and disappear very rapidly. There is ample reference in the pedological literature to get these concepts straight.

Albrecht: By the use of proximate analysis methods on the organic matter in the soils of Sanborn Field at the University of Missouri that have had different mineral treatments, it has been found that the deficiency of minerals—as shown in crop yields—also reflects itself as a deficiency in the microbial ration for more complete breakdown of the soil organic matter. Soils from fields not given calcium give increased carbon dioxide, nitrate, etc., when given calcium in the laboratory over those obtaining this as field treatment. Similar laboratory results are shown in the case of phosphorus, and in some cases for potassium.

These results suggest that chernozem organic matter may be in a high degree of microbial digestion, due to complete satisfaction of the microbial ration with reference to the bases or minerals. Chernozem is formed under little leaching, hence is well supplied with bases needed by microorganisms. It is not deficient in calcium, etc., for organic matter digestion, as seems to be true in many soils where organic matter "accumulates," particularly in such forms as will be further digested if supplied with some mineral nutrients, especially those giving increased crop yields when added to the soil. Thus in thinking of organic matter digestion within the soil, attention may well go to mineral deficiencies in the microbial relation.

Relative to the "stable humus," it is difficult to think of a "stability" in organic matter compounds except in their order of "solubility" or "reactivity" or "digestibility" in different chemical reagents. Microbial digestion is a chemical performance, and chemical properties of the compound control its digestion. If stability is a characteristic of organic compounds remaining in the soil after there is no further microbial attack, we might imagine a chemical composition approaching crude oil, with no oxygen, or no weak linkages in the configuration. The idea of much "stable humus" in the soil seems to have little place in our thinking when the legion of microbial changes in the soil are remembered.

Morgan: Consideration should be given to the physicochemical properties of what we have considered "humus," as a reasonably stable contribution to base-exchange properties of soils. The highly resistant colloidal humus isolated from peat by acid hydrolysis, precipitation with alkali, and subsequent electrodialysis is consistent in its performance with respect to base-exchange reaction, acid capacity, and is capable of being measured with respect to molecular weight, as by rate and diffusion through a silica membrane. To a soil physical chemist, perhaps this represents what Dr. Scheffer has designated "true humus." Surely its presence is exceedingly important in soil fertility.

Hofer: It is possible that some of the compounds usually regarded as stable are still capable of being decomposed. For instance, Dr. Hagan of Cornell University has found paraffin-utilizing bacteria in soil. He places a pebble in a solution of mineral elements, sterilizes this, and spreads a thin layer of sterilized paraffin upon the surface of the pebble which projects just above the surface of the solution. After a period of incubation growth of paraffin-using bacteria occurs on the pebble.

S. C. Vandecaveye: Microbial Activity in Relation to Organic Matter Transformation in the Soil.

Discussion

Batchelor: Dr. Vandecaveye should be congratulated on this work. As he has already indicated in his previous contributions as well as in the present paper, he is fully aware that "a rapidly fluctuating microbial population is only partially represented by plate counts." In spite of this he has been able to show some general positive relations between numbers of microorganisms in the soil and the different factors studied. In some cases, however, the data show either no correlation or relations inverse to those that might be expected. Although such inconsistencies generally develop in data obtained by the plating method of counting soil microorganisms their significance has been overlooked. The basic principles involved in the method of dispersing and sampling soil suspensions have been studied in considerable detail by physical chemists and soil physicists but for the most part appear to have been ignored by soil biologists. Until we apply these principles to our problems in soil biology we can hope to make little progress. At our meeting in New York next Monday, I hope to discuss this in more detail.

Charles E. Skinner: The Occurrence in Soil of Bacteria, Actinomycetes, and Molds Capable of Transforming Tyrosine to Melanin or Other Pigments.

Discussion

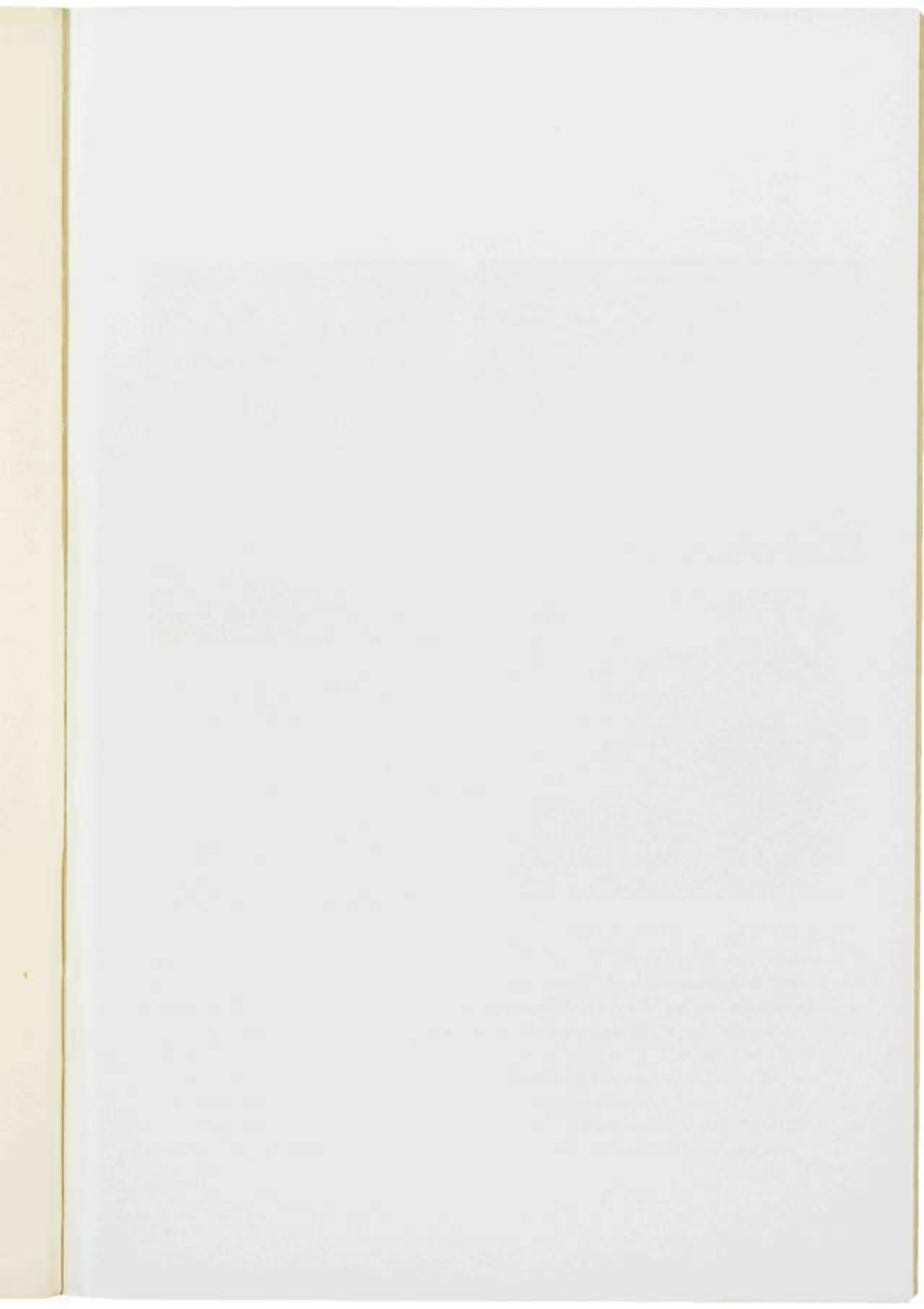
Stapp: Wenn nur zwei Typen von sporenbildenden Mikroorganismen beschrieben sind, von denen die erste Gruppe mit Tyrosin eine Rosafärbung, die andere sofort eine Dunkelfärbung hervorruft, so dürfte diese Reaktion allein auf der verschiedenen starken Aktivität der Tyrosinase beruhen, die in der zweiten Gruppe stärker ist als in der ersten. Wenn eine Bakterienaufschwemmung der ersten Gruppe in einer wässrigen Tyrosinlösung mit Chloroform geschüttelt und in ein Wasserbad von 45°C. gestellt wird, so tritt eine starke Beschleunigung in der Reaktion ein, und es kann geschehen, dass sich die Braun- bis Schwarzfärbung innerhalb einer oder weniger Minuten ein-

stellt. Der Unterschied ist also auf festen Substraten nur graduell. Interessieren würde mich noch zu erfahren, ob die genannten Organismen ihre Fähigkeit, Tyrosin in Melanin umzuwandeln nach längerer Züchtung auf künstlichen Substraten behalten haben, jedenfalls ist die Tyrosinasewirkung bei von mir isolierten Knöllchenbakterien stark zurückgegangen.

Chodat: Asked among other things if the difference between the red and the black pigment could not have been due to differences in conditions.

Skinner: From the same batch of medium, tubes inoculated with one organism gave a gray pigment turning black, and with the other, a pink turning red, and finally after many weeks, red-brown.

Stapp: Stated that he thought the red and black pigments were the same and that the black pigment was only a more intense red.





Photos by R. H. Burris, H. A. Wilson and W. B. Sarles

Upper left. Left to right: Dr. Dean Burk, Dr. S. F. Snieszko, Dr. I. L. Baldwin.
Upper right. Left to right: Dr. A. G. Norman, Dr. Norman James, Dr. R. E. Buchanan, Dr. F. B. Smith.
Lower left. SEEN ON THE EXCURSION. Left to right: Dr. A. G. Norman, Dr. Charles Thom, Dr. S. A. Waksman, Dr. F. Scheffer, Mr. D. M. Goss.
Lower right. Dr. E. B. Fred, Dr. S. A. Waksman, Dr. R. L. Starkey.

THURSDAY, AUGUST 31, 2 P.M.

Chairman: Dr. C. Stapp

Secretary: Dr. I. L. Baldwin

REPORTS ON AZOTOBACTER AND ITS SIGNIFICANCE
IN SOIL PROCESSES

Robert L. Starkey: The Influence of Reaction upon the Development of an Acid-Tolerant *Azotobacter*.

Discussion

Stapp: Die Ausführungen von Dr. Starkey sind sehr interessant, vor allem in Hinsicht auf die hohe Säureresistenz. Denn es gelingen sollte, diesen Organismus allen Kulturböden anzupassen, so wäre das vom praktischen Standpunkte sicher ein Gewinn. Auch uns ist es möglich gewesen, einen Organismus zu finden, der Stickstoff bindet und recht hohe Säurekonzentrationen verträgt, aber nicht zu dem genus *Azotobacter* gehört. Er hat ausserdem den Vorteil dass er eine sehr grosse Zahl von C- verbindungen verwerten kann. Ich werde darüber in New York anlässlich des Mikrobiologen-Kongresses berichten.

Thornton: *Az. indicum* was apparently found in paddy soils which are usually flooded. It might therefore be rather a water than a soil organism. It might be interesting to compare it with *Azotobacter agile* which has been isolated from the Dutch canals by Beijerinck and Kluver, and from fresh water in the English Norfolk Broads by Nicol. Both organisms appear to be normally flagellated, in contrast to other *Azotobacter* sp. in which flagella are uncommon. It might be worth comparing the acid tolerance of *Az. agile* with that of *Az. indicum*.

Starkey: I have previously suggested the possibility that *Az. indicum* is closely related to *Az. agilis* in view of the fact that it produces no cysts. This possibility is not excluded, since the organism has never been cultivated upon alcohols, which have been suggested by Winogradsky as most favorable for cyst formation. It will be recalled that *Az. agilis* and *Az. vinelandii*, essentially water forms, were separated from the soil forms on the basis of cyst production, among other characteristics. In view of the fact that *Az. indicum* was obtained from paddy rice soils, as stated, there is justification for the idea that water forms might be recovered from these soils. The data at hand as to the relationship to the water forms are no more than suggestive.

Burk: I would not ask if this organism just described is *Azotobacter*, but I would like to ask you, Dr. Starkey, this, "Is there any doubt in your mind that it is *Azotobacter*?" Perhaps suggestive of this is, first, the entirely different pH relations, which in all other described *Azotobacter* are so highly characteristic—almost unique—and about as unusual as the ability to fix nitrogen itself. Then there is the entirely unusual fat globule morphology. Also the rate of growth is so *very* slow compared with all other *Azotobacter*. So I would ask, does any doubt lurk in your mind that this may not be *Azotobacter*?

Starkey: Before making an answer as to whether or not the organism should seriously be considered as a member of the genus *Azotobacter* it would be necessary to characterize the genus. Briefly it might be assumed that the members are strictly aerobic nonsporulating bacteria, are commonly motile, form cysts in some cases, utilize lower carbohydrates, assimilate inorganic nitrogen, are generally unfavorably affected by many organic nitrogenous compounds, and commonly fail to grow upon them or in their presence. Above all, they have the capacity of utilizing molecular nitrogen in the absence of available fixed nitrogen. If these criteria are valid, I can see no reason for excluding the organism from the genus *Azotobacter*. Certainly there would appear to be no reason for excluding it merely on the basis of acid tolerance. There are organisms in the genus *Sulfomonas* which, in one case, cannot develop at neutrality or in alkaline media but grow well to below pH 1.0; on the other hand, some species fail to grow much below pH 6.0 and develop well at reactions above neutrality. I find no reason for excluding the organism from the genus *Azotobacter* and find many reasons for including it in this genus.

Stapp: Es dürfte nach meiner Meinung nicht schwierig sein, ein Bakterium als zu dem Genus *Azotobacter* gehörend zu identifizieren.

Herbert W. Reuszer: The Effect of Benzoic Acid Compounds upon the Abundance of Microorganisms, Including *Azotobacter* Organisms, in a Soil.

Discussion

Thornton: It is of course well known that many soil organisms can use aromatic compounds, such as phenol, cresol, and naphthalene as the sole source of their energy. I should be interested to know whether Dr. Reuszer has tried to isolate any organisms, by enrichment culture methods, that can use benzoic acid as their only energy source, and also to what extent *Azotobacter vinelandii* can utilize benzoic acid with no other source of energy.

Reuszer: No attempts have been made to isolate organisms utilizing only benzoic acid as a source of energy. Our investigations show that all strains

of *Azotobacter vinelandii* tested grow in a medium containing 1.5 per cent sodium benzoate and that many strains will tolerate as much as 2 per cent of the compound. No *Azotobacter chroococcum* strains were found capable of growing at concentrations of sodium benzoate above 1 per cent, and many of them will not grow above about 0.5 per cent.

G. Guillonnet: Sur l'utilisation des composés phenoliques comme aliment énergétique par les *Azotobacter* du sol (presented by Dr. Dufrenoy).

C. Kenneth Horner and Dean Burk: The Nature and Amount of Extracellular Nitrogen in *Azotobacter* Cultures.

A motion picture of Prof. S. N. Winogradsky, taken by Dr. S. A. Waksman on September 13, 1938, at Brie-Comte-Robert, was shown.

S. Winogradsky, *Institut Pasteur, Paris*: Sur la synthèse biogène de l'ammoniac dans le sol et les eaux (English translation read by Dr. Baldwin).

Dans une note à l'Académie des Sciences, séance du 19 Mars 1930, j'ai établi le fait de la production d'ammoniac par les *Azotobacter* dans un milieu privé d'azote combiné. A condition que le pH soit au-dessus de 8.0, la libération de l'ammoniac est déjà sensible aux tous premiers débuts de la végétation. Cette libération augmente rapidement, en atteignant son maximum à mesure de l'épuisement de la matière énergétique. En baissant lentement, elle dure des semaines et des mois.

La pensée physiologique se refusant à admettre un processus de décomposition dans les conditions données, la conclusion s'imposait que l'ammoniac est un produit intermédiaire de la fixation, qui devient stable, aussitôt que la végétation se ralentit ou s'arrête, faute d'aliment.

Cette conclusion a soulevé des critiques, qui lui reprochaient de ne s'appuyer que sur des données qualitative (Dean Burk et col.). En général elle n'a pas attiré l'attention qu'elle paraît mériter.

Pour écarter les objections concernant l'origine de l'ammoniac dégagée, il importait de chercher à déterminer le bilan de l'azote dans les cultures en question, ce qui serait possible au moyen de trois dosages successifs, à savoir: dosage de l'azote fixé par les cellules, dosage de l'azote exhalé sous forme d'ammoniac, enfin, dosage de l'azote fixé restant.

On s'est servi de plaques de silico-gel préparées selon notre méthode, pourvues de faibles doses d'éthanol ou de butyrate,ensemencées par des souches d'*Azotobacter*, isolées tout récemment du milieu naturel et n'ayant pas passé par les milieux standard à glucides.

Dans toutes les expériences, la moitié du lot des plaques est soumise à la Kjeldahlisation, d'après la micro-méthode de Pregl, mais en nesslerisant le

distillat, au lieu de le titrer; celà, au bout de quelques jours, aussitôt que l'aliment est consommé. L'autre moitié sert à l'extraction de l'ammoniac, au moyen de courtes distillations à basse température, répétées aussi souvent que possible, au cours de la longue suite de semaines et de mois que dure cette exhalation. Il va de soi, que les chiffres ainsi obtenus ne représentent pas la production totale de l'ammoniac, mais seulement la part que l'on a réussi à recueillir; les grosses pertes, inévitables avec un procédé quelque peu primitif, ainsi que causées par l'exhalation spontanée au cours du long séjour à 30°, étant impossible à évaluer. On a soin, bien entendu, d'ajouter de petites quantités d'eau distillée sur la surface du gel, autant que nécessaire pour prévenir la dessiccation. L'expérience est terminée par le dosage de l'azote fixé restant dans ces dernières plaques. Les résultats sont notés en gammas.

Voici les résultats de quatre expériences qui ont duré respectivement 160, 78, 98, 95 jours. On trouve par plaque:

Expériences	1	2	3	4
Azote fixé initial.....	9100	1040	2100	1141
Azote ammon. libéré.....	5771	870	1496	6039
Azote fixé restant.....	5110	630	980	—

Il est à remarquer, que ce n'est que dans la dernière expérience que l'on a appris à recueillir beaucoup plus d'ammoniac, en multipliant au possible les distillations, sans arriver pourtant jamais à épuiser le dégagement; tandis que, dans les trois premières, les distillations n'ont été poursuivies que de temps en temps, et l'exhalation y était encore bien en train, quand on les a soumises l'analyse final.

Il apparaît de ces expériences que la quantité de l'azote dégagé sous forme d'ammoniac est toujours supérieure à la différence entre l'azote fixé initial et l'azote fixé final. Mais là, où l'extraction a été poussée assez loin, le rendement en azote ammoniacal est énormément supérieur à celui de l'azote fixé par les cellules. Ce fait conduit nécessairement à la conclusion, que *l'azote exhalé ne peut avoir que l'atmosphère comme source*; autrement dit, *qu'il s'agit là bel et bien d'ammoniac synthétique*.

La nature enzymatique du processus ne peut être sujet à aucun doute, vu que la libération est accompagnée d'autolyse des cellules, qui conduit à leur entière désorganisation, jusqu'à ce que l'on ne réussit plus à déceler aucune cellule viable dans une culture en pleine production d'ammoniac.

La question se pose, aux dépens de quelle substance s'exerce la déhydrogénation qui fournit l'hydrogène nécessaire à la synthèse de l'ammoniac?

On n'en voit pas d'autre que la substance même des cellules. Ce qui frappe

dans ce cas, c'est le fait qu'une dose minime de substance énergétique—telle qu'un dixième de cc. d'éthanol, offerte dans l'expérience 4^{me}—peut donner lieu à un processus de synthèse de si longue durée et d'un rendement relativement important. L'azote étant disponible en quantité illimitée, c'est évidemment de la source d'hydrogène qu'il s'agit de se faire une idée, au point de vue quantitatif. Il semble possible d'y parvenir, en utilisant les données analytiques sur la composition élémentaire des cellules *Azotobacter*. En choisissant celles d'Oméliansky et Sieber,¹ d'après lesquelles la matière sèche des cellules contient 2.2% d'azote et 6.4% d'hydrogène, on prendra l'azote fixé par les cellules comme base de calcul, pour déterminer la quantité de substance produite et la réserve en hydrogène qu'elle contient. Pour l'expérience no. 4, on trouvera, par exemple, 3315 gammas d'hydrogène, ce qui pourrait suffir à hydrogéner en ammoniac une quantité cinq fois plus grande d'azote atmosphérique. S'il en est ainsi, la quantité maxima d'azote ammoniacal que nous avons réussi à extraire serait encore bien inférieure à la moitié de la réserve en hydrogène que contenait la substance des végétations.

Il résulte de ces expériences que la synthèse de l'ammoniac peut marcher dans un milieu minéral aux dépens de très faibles doses de substances dégradées, telles que l'éthanol ou les sels d'acides gras, en accumulant par une action lente, mais incessante, un rendement relativement important d'ammoniac synthétique.

C'est ce qui a lieu dans le sol, milieu parfaitement comparable, au point de vue oecologique, à nos plaques.

C'est ce qui a lieu également dans les eaux, où ce sont les *Azomonades* (syn. *Azotobacter agilis*), espèces exclusivement aquatiques, qui s'en chargent.

¹ Hoppe-Seyler's Ztschr. Physiol. Chem. 88:445-459 (1913).

THURSDAY, 4 P. M.

Business meeting, Dr. Thornton presiding

Dr. Gieseke, Berlin, berichtet über das vorläufige Programm der im Jahre 1940 in Deutschland stattfindenden Hauptversammlung der Internationalen Bodenkundlichen Gesellschaft. Der Tagungsort ist Heidelberg. Die Haupttagung beginnt Mitte Juli und dauert acht Tage. Der genaue Termin und das ausführliche Programm werden rechtzeitig bekannt gegeben. Die Haupttagung in Heidelberg ist von zwei grösseren Excursionen umrahmt. Die erste Excursion beginnt am 3. Juli in Hamburg und führt durch Nord und Nordwestdeutschland (Hochmoorgebiete) nach Heidelberg. Die zweite Excursion schliesst unmittelbar an die Tagung in Heidelberg an und führt über München, Salzburg, Wien, Nürnberg, Dresden nach Berlin. Von den genannten Städten aus werden die verschiedensten bodenkundlichen und landwirtschaftlichen Teilexcursionen unternommen.

Ich überbringe im Namen aller deutschen Kollegen die herzlichste Einladung an alle Anwesenden und spreche dabei die Bitte und die Hoffnung aus, eine sehr grosse Zahl der ausländischen Kollegen im Jahre 1940 in Deutschland herzlichst begrüßen zu können.

Resolutions Committee Reported by Dr. Thom

The members present at the New Brunswick meeting of the Third Commission, urgently recommend a change in the publications of the International Society of Soil Science for the purpose of conserving the limited funds available. To that end, dropping the present journal (the "pink journal") is recommended.

It is the opinion of the Third Commission that the available funds of the Society should be used to aid local committees in the publication of the proceedings of interim meetings of the several commissions. The present practice of forcing the local committees in charge of these important meetings to obtain funds necessary for such publication from local sources, puts an undue burden upon such committees and tends to discourage holding these very desirable meetings in countries in which such meetings are greatly needed. It is believed that such use of funds would be much more valuable to the Society than continued publication of the "pink journal." A copy of this resolution to be forwarded to the Honorary General Secretary: Dr. D. J. Hissink, International Society of Soil Science.

Committee:

E. B. FRED
JAN SMIT
CHARLES THOM

This resolution was unanimously adopted.

The members of the Third Commission of the International Society of Soil Science and of the Soil Science Society of America assembled at New Brunswick, August 30, 31, and September 1, 1939, extend their thanks to the local committee from New Brunswick and vicinity, to the President and Trustees of Rutgers University, to the Director of the New Jersey Agricultural Experiment Station, to the Princeton Laboratories of the Rockefeller Institution, and to the Walker-Gordon Dairies, for the many courtesies enjoyed during our stay in the state.

Committee:

E. B. FRED
JAN SMIT
CHARLES THOM

ROUND TABLE DISCUSSION ON LEGUME INOCULANTS

Methods of Testing Legume Bacterial Strains or Inoculants for Efficiency Chairman—A. W. Hofer

The discussion was divided into two main parts, namely, technique for use in field tests and methods for use in greenhouse tests of nitrogen-fixing efficiency of legume bacterial strains or inoculants.

In regard to field tests, there was agreement as to the need for a sufficient number of replications (at least three) to give an accurate measure of the performance of the bacterial culture under test. The desirability was suggested also of using more than one of the plant species or varieties for which the culture was said to be good. On the other hand, the fact was recognized that, many times, the extensiveness of the tests is limited by considerations of time, available labor, or land.

As to the type of soil to be used, a well-drained soil of favorable pH and suitably fertilized was preferred. Dr. Sarles, of Madison, Wisconsin, felt that there might not be as much objection as had commonly been supposed to the growing of a legume plant for bacterial testing on land where that legume had been grown before. He reported that isolations from such soils frequently consist of strains that fix nitrogen indifferently or poorly. On the other hand, a few (25) soils were found to contain native strains of legume bacteria that were efficient in nitrogen fixation. 90

Planting of seed can best be accomplished by a small seeder. This may be disinfected very simply. Weed control is by ordinary methods.

If the replicate rows (about 1 rod) are scattered at random over the plat, bacterial spread from one row to another may not occur; on the other hand, cases were observed where some spread had occurred.

The harvesting of such rows offers no particular difficulties. After drying, the plants should be ground to pass through a 40-mesh screen. If differences

in dry weight are large, nitrogen determinations may not be necessary. Where such differences are nonexistent or small, nitrogen (Kjeldahl) analyses are advisable, since differences in nitrogen fixation would be evident only as differences in percentage nitrogen in the plants.

The greenhouse tests were discussed briefly. In general, the same principles are to be observed in the greenhouse as those mentioned for the field. A number of replications should be made, and more than one species or variety of host plant should be tested from the group which the culture is said to inoculate. Nitrogen analyses are run as above, except that the plant roots also are dried, ground, and analyzed for this element.

A very constructive suggestion was made by Dr. H. W. Batchelor, of Wooster, Ohio, in regard to the question of pest control in the greenhouse. He said that if the nutrient solution contains 2 p.p.m. of selenate, the plants will not be bothered by red spiders; the sodium selenate is the salt used.

The meeting closed with a brief discussion of the possibility of using quick tests for efficiency. Mr. Leonard has reported the use of Ridgway's color standards as very helpful, inasmuch as the high-nitrogen plants have a much deeper green color than the low-nitrogen plants.

EXCURSIONS

Thursday, August 31, from 12:15-2:00 p.m., the participants of the conference visited the Squibb Institute at New Brunswick, N. J.

Friday, September 1, was devoted entirely to excursions through central New Jersey:

9:00 a.m. Visit to Princeton University.

10:00 a.m. Visit to Rockefeller Institute for Medical Research.

12:00 noon Visit to Walker-Gordon Laboratories.

1:30 p.m. Visit to Japanese Beetle Control Laboratories at White Horse where the parasitic nematodes are cultivated.

3:00 p.m. Visit to Soil Conservation Farm of Mr. George Dold where strip cropping, terraces, pasture furrows, and forest planting were examined.

4.00 p.m. Visit to Cranberry and Blueberry Substation at Whitesbog, N. J.

Saturday, September 2. A small group of participants visited the Soil Fertility plots, Agronomy plots, Plant Physiology Laboratories, and Horticulture Farm of the New Jersey Agricultural Experiment Station. Another group visited the Soil Conservation Station at Marlboro, N. J., and Soil Conservation Demonstration Farms.

NORTHEASTERN STATES SOIL TESTERS' CONFERENCE

THE day preceding the meetings of the Third Commission, August 29, a conference was held under the direction of Mr. D. M. Goss of the New Jersey

Agricultural Experiment Station for the consideration of rapid soil tests for plant nutrients.

There were 57 in attendance, including 21 representatives of fertilizer companies, 10 representatives from out-of-state agricultural experiment stations, 13 staff members of the N. J. Agricultural Experiment Station, 3 representatives from the U. S. Department of Agriculture, 5 farm managers, 2 visitors, and 1 representative from each of the following organizations: Quality Lime Institute, National Fertilizer Association, American Potash Institute.

The discussions of certain pertinent subjects were led by the following persons: Dr. Emil Truog, University of Wisconsin—the role of certain soil constituents in holding plant nutrients, their utilization by plants and the treatment of soils for their estimation; Dr. M. F. Morgan, Connecticut Agricultural Experiment Station—the nature and merits of various procedures for testing soils for plant nutrients; Dr. R. P. Thomas, University of Maryland—the evaluation and interpretation of results; Dr. Jackson B. Hester, Campbell Soup Co., Riverton, N. J.—the role of soil organic matter in crop production. Matters relating to sampling of soil were also discussed in some detail.

EXHIBITS

THERE were three groups of exhibits, dealing with peat, library material, and soil science.

The peat exhibit, arranged by the staff of a W.P.A. project for surveying the peat resources of New Jersey, included profiles of New Jersey peats; peat-forming plants; samples of peats of the world; sketches of peat areas; wood, concretions, and other materials recovered from peats; proximate analyses of peats, cultivated plants, and soil organic matter; and materials illustrating the utilization of peat.

The library exhibit comprised numerous old books on agriculture (published prior to 1800); books, papers, and other publications on soils by the staff of the New Jersey Agricultural Experiment Station during its fifty-nine years of existence; and notes and manuscripts by the first three directors of the New Jersey Agricultural Experiment Station, all of whom were soil scientists.

The soil science exhibit was devoted to various phases of soil microbiology and other branches of soil science. The following groups collaborated:

Division of Soil Microbiology. The soil population as revealed by the contact slide technique; sulfur transformations by some sulfur bacteria and other soil bacteria; an acid-tolerant *Azotobacter*—*Az. indicum*; soil actinomycetes; the production of organic acids by species of *Rhizopus*; bind-

ing and aggregation of soil particles by microorganisms; plaques of bacteriophage active on *Rhizobium meliloti*.

Division of Soil Chemistry and Soil Fertility. Results of cropping and fertilization of New Jersey soils; soils of New Jersey; soil map of New Jersey; rapid soil testing service.

Department of Plant Pathology. Microbial antagonism.

Department for the Control of Seeds and Legume Inoculants. Legume inoculants in New Jersey.

Soil Conservation Research Station. Measurement and control of soil moisture; the role of organic matter in soil conservation.

U. S. Soil Conservation Research Service. The problems and control of soil erosion in New Jersey.

Federal Laboratories for the Control of the Japanese Beetle. Control of soil-infesting larvae of the Japanese beetle by soil-borne microbial parasites.

Department of Plant Pathology of the Rhode Island Agricultural Experiment Station. Soil fumigation with chloropicrin.

