NATIONAL ACADEMY OF SCIENCES

CHESTER HAMLIN WERKMAN

1893—1962

A Biographical Memoir by

RUSSELL W. BROWN

Biographical Memoir

CHESTER HAMLIN WERKMAN

June 17, 1893–September 10, 1962

BY RUSSELL W. BROWN

CHESTER HAMLIN WERKMAN broadly conceived of his sphere of scientific interest as physiological microbiology. His career, which extended over a period of approximately forty years (1921–1962) was spent for the most part in the investigation of the intermediate steps by which microorganisms accomplished the biochemical transformations which enabled them to obtain energy for their essential role in nature. During that time he was one of a relatively small group of microbiologists in the United States and in Western Europe whose primary concerns were to understand and reconstruct the specific enzymatic pathways involved in metabolic processes. The anaerobic and microaerophilic bacteria were of immediate interest because their chemical transformations were more readily quantitated and expressed by carbon and oxidation–reduction balances of the products. With these organisms it was possible generally to conduct experiments in which known quantities of substrates were converted to intermediate and end products which were determined quantitatively. A variety of quantitative methods and experimental procedures were developed which made it possible to greatly expand the knowledge of the biochemistry of microorganisms. Werkman and his students at Iowa State University and a group at the University of Wisconsin were perhaps the most productive investigators in the field of microbial biochemistry in the United States during the 1930s when work elsewhere was oriented primarily toward the morphology and the pathogenic activities of microorganisms. There were, concurrently, the well-known investigations of A. J. Kluyver and his associates in Holland and of a number of other outstanding biochemists and microbial physiologists in Europe, including such names as G. Embden, H. von Euler, H. A. Krebs, Fritz Lipmann, Otto Meyerhof, Carl Neuberg, J. H. Quastel, Marjorie Stephenson, T. Thunberg, A. I. Virtanen, Otto Warburg, H. Wieland, and others. Present-day cell biology, and more specifically molecular biology, is concerned with the interrelationships of a multiplicity of substances within the cell. Moreover, in more recent years methods and procedures have advanced tremendously beyond those that were in use during Werkman's life. Nevertheless, the investigative approach employed by Werkman and his contemporaries was in large measure the foundation upon which the present-day concepts of bacterial metabolism were built.

To some extent, fortuitous circumstances may have played an important role in the life of Chester Hamlin Werkman. He was a graduate student at Iowa State University during the early twenties under Dr. Robert Earle Buchanan, who had already achieved national and international recognition in the systematic and general aspects of microbiology. When Werkman became associated with the university, the Department of Bacteriology in the Division of Science was making a concerted effort in developing a research program; he entered, therefore, an academic situation in which the climate was favorable for a person of unusual energy and aptitude for research. His career was launched during a period when some investigators were beginning to examine with vigor the biochemical aspects of microbial activity. His undergraduate concentration in organic chemistry enabled him to view the microbe, not as the ultimate object of interest, but rather as a versatile tool for the investigation of the chemical activities of living organisms. It can be said with certainty, however, that fortuitous circumstances were not the most important factors in the career which he achieved; perhaps more than anything else his entire life was motivated by a strong desire to be involved at the frontier of his field, and this ambition was reinforced by an unusual store of energy and personal drive.

Werkman was born in Fort Wayne, Indiana, June 17, 1893, and died in Ames, Iowa, September 10, 1962. He is survived by his wife, Mrs. Cecile Werkman, and their son, Robert, who has been a resident for a number of years of Bartlesville, Oklahoma, as well as by a sister, Mrs. Iona Werkman Leonard, of Fort Wayne, Indiana. Very little information of record is available regarding Werkman's early life. He did not have much inclination to discuss his boyhood with family and friends and on only very rare occasions did he reminisce about his elementary and high school experiences in Fort Wayne and later as an undergraduate student at Purdue University. Nevertheless, the following information, resulting primarily from Mrs. Werkman's recollections, gives some knowledge of the period when Werkman, as a young boy, found it necessary to assume responsibility for his livelihood and to chart his own future without the aid of strong family ties. These recollections offer some insight certainly into the nature and quality of the motivations which shaped his character and personal outlook, and which doubtless were responsible for his ambition to achieve recognition as a scientist in later years. The statements which follow have resulted also from recollections by Dr. Buchanan of conversations with Werkman, both during the period when the latter was a graduate student and during the subsequent years of their association at Iowa State.

Werkman's parents were of English, German, and Dutch descent; his mother was a schoolteacher before she was married; his father operated a barbering establishment and was active in the affairs of the barber's union in Fort Wayne. Werkman's mother died when he was age fourteen or fifteen and the author is uncertain when his father died; it is assumed, however, that this event occurred perhaps about the time of Werkman's first year as an undergraduate student at Purdue University.

Werkman attended secondary school at Fort Wayne, where he excelled in his studies. His performance in chemistry was especially outstanding and during his senior year he served as laboratory assistant to the instructor. He was permitted to do independent experimentation and promptly had an explosion, thus learning the hard way some of the hazards involved in the chemical laboratory. As a consequence of this accident he was unable to attend the graduation party because of burned hands. After graduation from high school he was employed by the Pennsylvania Railroad at Fort Wayne, Indiana; interestingly, his superintendent in this job was influential in persuading him to attend college.

He had saved some money from working and he entered Purdue University as an undergraduate. His undergraduate major was organic chemistry and the academic record which he made at Purdue was of the highest order; his grades were all A's except for one B. He applied himself diligently in the formal class and laboratory with a tenacious desire to excel; he despised being second best in anything. During the summer between his junior and senior years at Purdue, he worked for the federal government in what is now called the Pure Food and Drug Administration in the Department of Agriculture and was involved in the inspection of carloads of eggs for contamination and spoilage.

After his graduation from Purdue University in 1919, Werkman moved to Philadelphia where he continued, for a brief period, the job of inspection of food products as an employee in the Department of Agriculture. He was unhappy, however, with the routine nature of this assignment and, after a very few months, he accepted a position as an instructor in chemistry at the University of Idaho. While there, he published his first research paper with W. M. Gibbs, "Tuberculosis of poultry," *Idaho Agr. Exp. Sta. Bull.* 126, 1921, which marked the beginning of a long list of scientific publications. Moreover, it is significant that during his brief stay at the University of Idaho, a second paper which he co-authored with Gibbs was published—"Effect of tree products on bacteriological activities in soil. I. Ammonification and nitrification," *"Soil Science,* 13:303–22, 1922. But the insight which he had gained into the nature and structure of the university made him aware that graduate study, with the attainment of the doctoral degree, was essential for his advancement in the academic community. He therefore began to investigate immediately the opportunities for a graduate fellowship. As a result of several applications he received awards from the University of Chicago, Iowa State University, and the University of California–Los Angeles. He decided to accept the fellowship at Iowa State, primarily because he thought that living expenses would be less in Ames than in the urban centers of Chicago and Los Angeles. He entered the graduate school at Iowa State in September 1920 in the Department of Bacteriology, with Dr. Robert E. Buchanan as his major professor.

The desire to excel was as much the driving force for Werkman's success as a graduate student at Iowa State as it had been during his undergraduate years at Purdue. He applied himself diligently and his background training in organic chemistry served him well in investigations in the field of immunology. He completed the requirements for the Doctor of Philosophy degree in the spring of 1923 and his thesis, entitled "Immunologic Significance of Vitamins," was published in the *Journal of Infectious Diseases* in 1923 in three parts:

Immunologic significance of vitamins. I. Influence of the lack of vitamins on the production of specific agglutinins, precipitins, hemolysins and bacteriolysins in the rat, rabbit and pigeon;

II. Influence of lack of vitamins on resistance of rat, rabbit and pigeon to bacterial infection;

III. Influence of the lack of vitamins on the leukocytes and on phagocytosis.

Subsequently, two additional publications appeared in this initial series:

Werkman, C. H., V. E. Nelson, and E. I. Fulmer. Immunologic significance of vitamins. IV. Influence of lack of vitamin C on resistance of the guinea-pig to bacterial infection, on production of specific agglutinins, and on opsonic activity. *J. Infect. Diseases,* 34:447–53, 1924.

Werkman, C. H., F. M. Baldwin, and V. E. Nelson. Immunologic significance of vitamins. V. Resistance of the avitamic albino rat to diphtheria toxin; production of antitoxin and blood pressure effects. *J. Infect. Diseases,* 35:549–56, 1924.

After receiving the Ph.D. degree, Werkman was still influenced by a restless urge to find an academic situation where he believed his desires and ambitions could be realized with some degree of dispatch. He remained with Dr. Buchanan at Iowa State during the academic year 1923–1924, and then he accepted a faculty position at the University of Massachusetts for one year, 1924–1925; but in the fall of 1925 he returned to Iowa State, where he became involved with the biochemical and physiological aspects of microbiology until his death in 1962.

Perhaps it can be said that Werkman's professional career had its real beginning in 1925 when he made the decision to return, once again, to Ames, Iowa. This move initiated his permanent association with the Department of Bacteriology at Iowa State and with Dr. Buchanan and another member of the department, Dr. Max Levine, who already was achieving recognition for his work in sanitary bacteriology. One of Werkman's early assignments was to assist Dr. Levine with routine water analysis and the bacteriological analysis of specimens from the college hospital.

During the period 1925–1930, Werkman developed an interest in the relatively unexplored field of the biochemistry of bacteria and began to stake out his own scientific domain. He often remarked that he did not envision as promising the conventional approach to the study of microorganisms which was mostly morphological and otherwise descriptive. He felt that the science had too long adhered to the conventional methods of the botanists and zoologists with not enough attention being given to the more complex biochemical role of microorganisms in nature. Werkman viewed bacteria primarily as the least complicated models for the study of the basic chemical transformations involved in living processes. He became interested in certain groups of bacteria because of their unusual fermentative activity, the end-products of their metabolic processes, and the mechanisms by which these products were produced from an initial substrate. For example, the genus *Propionibacterium* constituted one of his early interests, which he pursued for a number of years along with many other types of fermentative bacteria. The fact that the members of the genus *Propionibacterium* were responsible for the specific flavor and aroma of Swiss cheese was of little consequence; he was intrigued by the chemical sequence involving the quantitative transformation of glucose to propionic and acetic acids and carbon dioxide. As will be seen later, this particular brand of scientific curiosity led Werkman and his graduate students in numerous directions but always in pursuit of some aspect of the processes initiated by enzymatic activity.

In all fairness, it must be recorded that Werkman was endowed with an unusual capacity and perhaps an instinct for originality and innovation; he was in search constantly for a "new angle," either a completely new idea regarding the chemical activities of microorganisms or at least a novel approach to the most significant things being done at the moment by the most progressive investigators in other laboratories. Not only did he consider it essential to be active in the "mainstream" of bacteriological research, preferably enzymatic research, but it was most important that he and his students be in the vanguard. Moreover, Werkman had a very uncommon fascination for scientific equipment; a new and sophisticated item was not merely a research tool, it was also a thing of beauty. He was exceedingly adventuresome in his eagerness to undertake a novel approach to a particular research project, and he was especially interested in developing new research methods and procedures and in the further refinement of methods developed by other investigators. He was actually a gadgeteer in many respects. It frequently developed that new items of equipment were built in his laboratory long before they became available commercially.

Having had his basic training essentially in the field of organic chemistry, Werkman utilized consistently the chemical approach to the study of microbiological processes. He was concerned almost exclusively with the nature and mode of action of the enzymatic systems that enabled microorganisms to perform their role in nature. He viewed microorganisms as intriguing systems of enzymes capable of a multiplicity of chemical transformations, but with relative similarity in their basic biochemical behavior. His thinking was always foremost in the biochemical vein; he was most at home with chemists; and when he sought collaboration in research outside of his own laboratory at Iowa State, a review of his publications reveals that he almost invariably involved members of the faculty in the Department of Chemistry. Werkman had virtually no contact with undergraduate students, since he very rarely offered a course to undergraduate majors in bacteriology, and his graduate students, with few exceptions, had majored in chemistry as undergraduates. He was known to comment on numerous occasions that the student best qualified to pursue the doctoral program in his laboratory was one with basic training in chemistry, and that such a student could acquire the necessary acquaintance with microorganisms as they became useful to him in his biochemical investigations.

Publications bearing Werkman's name during the period 1920–1930 exhibit a variety of unrelated research efforts and suggest that he was searching for an area that would become his primary focus. These early adventures in research included items such as tuberculosis infection in poultry, ammonification and nitrification in soils, coli-aerogenes organisms in swimming pools, immunologic significance of vitamins, dye utilization in bacteriological media, continuous reproduction of microorganisms, accessory growth factors for microorganisms, bacterial spoilage of canned vegetables, synthesis of vitamins by microorganisms, and factors influencing the death rates of microorganisms. In 1922, however, two papers ("The production of propionic acid from pentoses by *Propionibacterium pentosaceum*," and "Physiological behavior of the propionic acid group of bacteria"), presented at a meeting of the Iowa Academy of Sciences, can be said, in retrospect, to represent the essential direction of his subsequent research interests.

Although he focused his attention on the chemical activities of many types of organisms, Werkman was interested also in their identification by the products of fermentation and the relationship of the several species of bacteria within a generic grouping. However, the bacteria of the genus *Propionibacterium* and the members of the genus *Clostridium* that produce butyl and isopropyl alcohols and acetone were the most thoroughly investigated by him. These bacterial species were examined systematically by the conventional procedures for classification, but more importantly they became the subjects of a long-range program of biochemical investigations.

The classification of the propionic acid group of bacteria was investigated extensively by C. B. van Niel during the period when he was associated with Professor A. J. Kluyver at Delft, Holland, and is described in his doctoral dissertation, *The Propionic Acid Bacteria,* published in 1928. Subsequently, studies of these bacteria were conducted in Werkman's laboratory resulting in some extensions and modifications of van Niel's classification and nomenclature and in the proposal for the establishment of the additional specific designation of *Propionibacterium raffinosaceum* (Werkman and Sara Kendall, 1931, "The propionic acid bacteria. I. Classification and nomenclature"). This work was based upon the conventional methods and criteria employed in the systematic study of nonpathogenic bacteria. At this time, eleven species had been recognized and Werkman was not satisfied that the classification of the genus *Propionibacterium* had been firmly established. It was his nature to be intrigued with the novelty in research methods and he encouraged one of his graduate students to apply serological procedures to the study of the propionic acid bacteria (Werkman and R. W. Brown, 1933, "The propionic acid bacteria. II. Classification"). This investigation of the genus *Propionibacterium* is perhaps the first instance in which serological procedures were employed in the study of nonpathogenic microorganisms, and it confirmed the genetic relationship of the several species of the propionic acid bacteria.

In directing his attention to the physiology of microorganisms, Werkman undoubtedly was influenced to a considerable extent by the work of Dr. Kluyver and his associates in the Delft laboratory. He developed great admiration for Kluyver; they visited each other's laboratories and they eventually became warm friends. Moreover, Werkman's interest in the propionic acid bacteria, as well as in other fermentative types of microorganisms, was stimulated further by the work of van Niel and other European investigators who at that time were making important advances in this highly specialized area of science.

During the decade 1930–1940, the involvement of Werkman and his graduate students in the mechanisms of bacterial fermentations was clearly established, and it was during this period that the Werkman laboratory made its initial impact as an important contributor in the field of microbial physiology. Attention was focused first on the quantitative determination of the end-products of the fermentation of carbohydrate substrates by various fermentative microorganisms including propionic acid, lactic acid, butyric acid–butyl alcohol, and coliform bacteria. In this endeavor it was considered essential to render an accurate account of the fate of the carbon substrate in the tradition of the quantitative organic chemist. Since many of the known procedures were not of sufficient accuracy, it was necessary to direct much of the effort of the laboratory toward developing more highly refined qualitative and quantitative methods. In 1930 Werkman applied the partition method in the determination of mixtures of fatty acids, and for several years thereafter he and his graduate students made significant advances in the development and refinement of quantitative methods. Especially noteworthy were the contributions of O. L. Osburn, Harland G. Wood, and Grant L. Stahly in the refinement and extension of the partition method, as well as the application of other quantitative procedures in the determination of a variety of the products of bacterial fermentations. These methods were concerned primarily with mixtures of compounds such as ethyl, isopropyl, and butyl alcohols; acetaldehyde, acetone, formic, acetic, propionic, lactic, pyruvic, and butyric acids, trimethylene glycol, 2,3-butylene glycol, acetylmethylcarbinol, diacetyl, carbon dioxide, and hydrogen.

During this period some of the research in Werkman's laboratory was sponsored in part by grants from industry, particularly corporations utilizing agricultural products in the production of the so-called organic solvents. It would be appropriate to list these corporations, but unfortunately accurate data are not available. Werkman made a specific effort to cultivate the acquaintance of research and administrative personnel in the fermentation industry and to enlist their interest in his program of research at Iowa State. While his personal commitment was to fundamental research mainly, he exerted considerable effort in applied research, including such things as: the fermentation of pentosans from cornstalks, production of acetic acid from cornstalks by thermophilic bacteria, production of trimethylene glycol by fermentation, lactic acid fermentation of malt sprouts, the utilization of agricultural byproducts in the production of propionic acid, fermentation of artichokes, determination of the furfural-yielding constituents of plant materials, and the utilization of agricultural products and wastes for production of butyl and isopropyl alcohols. He served as a consultant to several corporations in the fermentation industry and occasionally would assign some of his graduate students to stort-term exploration of some specific problem encountered in the industry.

Gradually, however, the research became increasingly more sophisticated in an effort to elucidate the intermediate mechanisms of microbial fermentations. The role of pyruvic acid as an intermediate was investigated extensively in a variety of types of fermentations and likewise the intermediate roles of various fatty acids, such as the condensation of two molecules of acetic acid to form butyric acid and the degradation of the four-carbon butyric acid to form acetone and carbon dioxide in the butyl alcohol fermentation. These investigations and others similar in nature projected Werkman and his students into the "mainstream" of scientific endeavor.

Perhaps it can be said that the early recognition of Werkman and his students came as a result of the innovations in their approach to investigations in microbial physiology. Although van Niel in 1928 postulated that pyruvic acid was formed during the fermentation of glucose by the propionic acid bacteria, it was not established as an intermediate product in the propionic acid fermentation until it was isolated and identified by Wood and Werkman and reported in 1934. This was accomplished by fixation of the keto-acid with sodium bisulfite, using the procedures devised by Neuberg to isolate acetaldehyde. Fixation with bisulfite was applied also in investigations of the intermediate roles of acetaldehyde and pyruvic acid in the fermentation of glycerol by bacteria belonging to the coli-aerogenes group, and the presence of formaldehyde as an intermediate compound in the fermentation of glucose by certain members of the genus *Propionibacterium* was detected by fixation with dimethyldehydroresorcinol (dimedon).

In 1933, G. Embden, H. J. Denticke, and G. Kraft, and Otto Meyerhof and W. Kiessling proposed phosphoglyceric acid as the key intermediate in yeast and muscle glucolysis, thus replacing methylglyoxal as an intermediate. Werkman and his students, being particularly alert to developments by the German investigators, became very interested in the intermediary role of phosphoglyceric acid. In a series of publications, R. W. Stone and Werkman (*Iowa State Col. J. Sci.*, 10:341–43, 1936; 11:1–3, 1936; *Biochem. J.*, 31:1516–23, 1937), Werkman, E. A. Zoellner, Henry Gilman, and Howard Reynolds (*J. Bacteriol.*, 31:5, 1936), and W. P. Wiggert and Werkman (*Biochem. J.*, 32:101–7, 1938) reported on the formation of phosphoglyceric acid and related intermediary compounds in the dissimilation of glucose by a variety of heterotrophic microorganisms, including *Citrobacter freundii, Escherichia coli, Propionibacterium arabinosum, P. pentosaceum, Aerobacter indologenes, Lactobacillus pentoaceticus, L. plantarum, Bacillus subtilis, B. mycoides, Azotobacter vinelandii, Streptococcus paracitrovorus, Staphylococcus albus,* and *Aerobacter aerogenes.* Although Neuberg and

M. Kobel (*Biochem. Z.*, 264:456, 1933) had shown that phosphoglyceric acid was converted to pyruvic acid and phosphoric acid by *Lactobacillus delbruckii;* Werkman, Zoellner, Gilman, and Reynolds (1936) were the first to isolate phosphoglyceric acid from bacteria. Moreover, it was Stone and Werkman (1936–1937) who demonstrated the general occurrence of phosphoric esters among members of the order *Eubacteriales.*

Perhaps the most important contribution which came from Werkman's laboratory was the demonstration in 1936 of the utilization of carbon dioxide in the dissimilation of glycerol by the propionic acid bacteria. While the research was done under Werkman's direction, it was the alertness of his associate and former student, Harland Wood, in interpreting the quantitative data obtained which demonstrated the utilization of CO_2 by heterotrophic bacteria, a supposition which Werkman was reluctant at first to accept. However, after the data were more carefully examined he readily supported Wood's interpretation that CO_2 was indeed utilized in the fermentation of glycerol by propionic acid bacteria. This demonstration of the synthetic use of carbon dioxide by heterotrophic bacteria gained for Harland Wood the Eli Lilly Award in 1942 and the focus of national and international attention on the research of the Werkman laboratory. In presenting their experimental results Wood and Werkman stated:

"The most significant fact shown by the data is the apparent utilisation of CO_2 by the propionic acid bacteria. This was evident, since the CO_2 at the conclusion of the fermentation was not equivalent to that of the original medium in the form of $CaCO_3$. This observation has been substantiated by two types of calculation of especial value, i.e., carbon recovery and redox index. If CO_2 is utilised, and is in turn (after synthesis) dissimilated, then calculations based on the assumption that glycerol is the sole source of carbon, should show an excess of products, i.e., the calculated recovery of carbon will exceed 100 per cent. The data presented show that this occurred and that calculations based on glycerol *plus* CO_2 are acceptable. The calculation of carbon recovery is not in all cases entirely satisfactory proof of CO_2 utilisation, but the oxidation–reduction balance is convincing. CO_2 contains but one carbon and requires a large utilisation to show a detectable change in the carbon balance; in the oxidation–reduction balance the CO_2 is highly oxidised and therefore has a marked effect. The data show that results calculated on the basis of glycerol *plus* CO_2 are reasonable and acceptable. The fact that the chemical analysis shows a decrease of CO_2 is perhaps proof enough of CO_2 utilisation. However, the carbon and oxidation–reduction balances furnish additional evidence.

"One important problem, which requires consideration in relation to the data presented, is the mechanism of succinic acid formation. A number of investigators working particularly with yeast and fungi (Butkewitsch and Federoff in 1930; Wieland and Sonderhoff in 1933) have reported that succinic acid is formed by a condensation of two molecules of acetic acid. Virtanen in 1925, and Virtanen and Karstrom in 1931, however, have suggested that the propionic acid bacteria produce succinic acid from glucose by a 4- and 2-carbon cleavage of the hexose molecule. Virtanen's proposal was prompted by the observation that the propionic acid bacteria in the presence of toluene form succinic and acetic acids from glucose with no gas. This observation appeared incompatible with schemes involving a 3-carbon cleavage and the formation of 2-carbon compounds by a 2- and 1-carbon cleavage. The absence of CO_2 or other 1-carbon compounds appeared conclusive proof against such a scheme. However, the present evidence of the utilisation of CO_2 by the propionic acid bacteria leaves no reason to assume that the 1-carbon compounds should equal the sum of the

2-carbon compounds. It is necessary in the light of our present knowledge to leave open the possibility of a 4- and 2-carbon cleavage."

Following the appearance of the publication by Wood and Werkman in 1936, it was not surprising that other investigators questioned the evidence for this "physiologically important phenomenon." For example, van Niel in 1937 made the significant statement that "Wood and Werkman claim that carbon dioxide is reduced during the fermentation of glycerol by propionic acid bacteria. The published results cannot, however, be considered conclusive, although the data do seem to favor their claim." A challenge of this nature issued by van Niel and others was an important stimulus to Wood and Werkman to present more experimental data in support of the utilization of carbon dioxide by heterotrophic bacteria. Werkman and Wood and their associates further extended investigations of the role of carbon dioxide in the dissimilation of carbon compounds by heterotrophic bacteria, and it was mainly through their efforts that the utilization of carbon dioxide became firmly established as an important component of biochemistry.

It has been inferred earlier that Werkman was fascinated by the most advanced and sophisticated equipment and he could always be depended upon to secure the funds necessary for new equipment, especially if the item was of such recent concept that it was not available commercially. Moreover, he was searching constantly for new and improved techniques for investigating the intermediate pathways of microbial fermentations. It was fortunate, therefore, that Alfred O. Nier in the Department of Physics, University of Minnesota, published the description of "a mass spectrometer for routine isotope abundance measurements" (*Rev. Sci. Instruments* 11:212–16, 1940) and suggested that the apparatus was sufficiently accurate to measure the $^{13}C/^{12}C$ ratio if separated ^{13}C was used as a tracer in biological investigations.

Measurements of the stable carbon isotopes by means of the mass spectrometer had revealed that 1.09 percent of the carbon in nature was ^{13}C, and in 1941 A. O. Nier and J. Bardeen published a paper on "the production of concentrated carbon (13) by thermal diffusion" (*J. Chem. Physics,* 9:690–92, 1941). They adapted the thermal diffusion method of K. Clusius and G. Dickel (*Naturwissenschaften,* 26:546, 1938; 27:148, 1939) for the concentration of $^{13}CH_4$ by constructing a 72-foot diffusion column broken into two parts in an available vertical space of approximately 36 feet. With this column it was possible to increase the ^{13}C content of methane from 1.09 to 11.5 percent.

In 1941, after consultation with Nier, Harland Wood supervised and assisted in the construction of a 72-foot thermal diffusion column in the elevator shaft of Science Hall and a mass spectrometer in the basement section of Werkman's laboratory. Both of these equipment items were constructed essentially in accordance with Nier's specifications and with the participation of the graduate students who were with Werkman at that time. This was a very substantial undertaking for a group with relatively little experience in the construction of scientific equipment, and this accomplishment placed Werkman's group in a very advantageous position by providing them with the immediate use of such highly sophisticated research tools. With the assistance of personnel in the Department of Chemistry, various ^{13}C-labeled compounds were synthesized and utilized as substrates in the study of metabolism.

The first detailed publications from Werkman's laboratory reporting the use of ^{13}C-labeled compounds appeared in the *Journal of Biological Chemistry,* 139, 1941. The authors of these papers were H. G. Wood, C. H. Werkman, Allen Hemingway, and A. O. Nier, and the articles represented a joint publication between the Bacteriology Section, Agricultural Experiment Station, Iowa State College, Ames, Iowa, and the Departments of Physiological Chemistry and Physics, University of Minnesota, Minneapolis. The first paper, entitled "Heavy carbon as a tracer in heterotrophic carbon dioxide assimilation" (1941), gave further confirmation to the earlier work of Wood and Werkman by demonstrating that $^{13}CO_2$ fixed in fermentations of galactose, pyruvic acid, or citric acid by *Escherichia coli* occurs exclusively in the succinic and formic acids, and that in the fermentation of glucose and glycerol by *Propionibacterium pentosaceum* the fixed carbon dioxide is in the succinic acid, propionic acid, and propyl alcohol. Moreover, "the data obtained by determination of the fixed ^{13}C are in agreement with the suggestion that succinic acid is formed by union of a 3-carbon compound and carbon dioxide and the propionic acid by decarboxylation of a symmetrical dicarboxylic acid containing fixed carbon dioxide in only one carboxyl group." Further evidence for the mechanism of succinic acid synthesis was presented in the second paper, which showed by degradation of succinic acid synthesized by *E. coli* and *P. pentosaceum* that the fixed carbon dioxide, which had been labeled with ^{13}C, occurred exclusively in the carboxyl groups of the acid. This finding was in agreement with the author's proposal that carbon dioxide is fixed by union with pyruvic acid. The same authors published a report (*J. Biol. Chem.*, 142:31–45, 1942) on the "fixation of carbon dioxide by pigeon liver in the dissimilation of pyruvic acid." These investigators had presented evidence earlier that in the case of a number of bacteria the initial reaction in the fixation of carbon dioxide proceeded as follows:

$$CO_2 + CH_3 \cdot CO \cdot COOH = COOH \cdot CH_2CO \cdot COOH$$

It was presumed that the oxalacetate was converted to other 4-carbon dicarboxylic acids, and it was of interest to determine whether animal tissues fix carbon dioxide by this reaction. The method employed was essentially a system by which "pyruvate was dissimilated by ground pigeon liver in a medium containing $NaHCO_3$ enriched with ^{13}C." In summarizing the experiments with pigeon liver the authors stated that "the dissimilation of pyruvate by pigeon liver occurs with accompanying fixation of carbon dioxide. By use of $^{13}CO_2$ the fixed carbon has been shown to be exclusively in the carboxyl groups of the 4-carbon dicarboxylic acids (malate, fumarate, and succinate), the carboxyl adjacent to the carbonyl of α-ketoglutarate, and the carboxyl of lactate. Aerobically in the presence of malonate succinate is formed which contains little or no fixed carbon. It is proposed that the 4-carbon dicarboxylic acids are formed by two mechanisms, one reductive through the carbon fixation reaction, the other oxidative by a tentative and modified Krebs cycle which does not involve citric acid.* The scheme accounts for the observed positions of the fixed carbon and the aerobic formation in the presence of malonate of succinate not containing fixed carbon." The results provided strong support for Krebs's proposed cycle.

H. D. Slade, Wood, Nier, A. Hemingway, and Werkman (*J. Biol. Chem.*, 143:133–45, 1942) investigated the extent to which other heterotrophic bacteria were capable of carbon dioxide fixation. They reported that "fixation of CO_2 by C_3 and C_1 addition is apparently a very general reaction," as demonstrated with several genera of heterotrophic bacteria, including *Aerobacter, Proteus, Staphylococcus, Streptococcus,* and *Clostridium.* In summary, they stated that "the assimilation of CO_2 is established as a general phenomenon among heterotrophic bacteria. It is shown by the use of heavy carbon, ^{13}C, as a tracer, that the fixed carbon is located in the carboxyl groups of succinic, lactic, and acetic acids. The assimilated CO_2 is distributed as follows: *Aerobacter indologenes,* acetate, lactate, and succinate; *Proteus vulgaris, Streptococcus paracitrovorus,* and *Staphylococcus can-*

* This conclusion was in error and was based on the idea that citrate is a symmetrical molecule and therefore the ketoglutarate formed from citrate was expected to be labeled in both carboxyl groups. Only when Ogston (Ogston, A. G., *Nature,* 162:4129, 1948) explained that an enzyme can distinguish between the primary carboxyl of citrate did this incorrect conclusion become clarified.

didus, lactate and succinate; *Clostridium welchii,* acetate and lactate; *Clostridium acetobutylicum,* lactate."

A heat-labile enzyme prepared from *Micrococcus lysodeikticus* was described by L. O. Krampitz and Werkman (*Biochem. J.* 35:595–602, 1941) which catalyzed the decarboxylation of oxalacetate with the formation of pyruvic acid and carbon dioxide, suggesting that the enzyme is involved in the reverse process of carboxylation, as was proposed by Wood and Werkman in CO_2 utilization. Subsequently, in experiments using $NaH^{13}CO_3$, Krampitz, Wood and Werkman (*J. Biol. Chem.* 147: 243–53, 1943) reported that "the exchange of $^{13}CO_2$ with the carboxyl groups of oxalacetic acid during spontaneous decarboxylation was found to be insignficant. The exchange, however, was increased significantly during the enzymatic decarboxylation of the acid. The exchange occurred exclusively in the carboxyl group adjacent to the methylene group." They concluded that "these results are further evidence of the validity of the fixation reaction as proposed by Wood and Werkman."

In the presence of glucose, washed cell suspensions of *Aerobacter indologenes,* which had been grown in nutrient medium containing peptone, glucose, and inorganic salts, were shown to condense two molecules of ^{13}C-labeled acetic to succinic acid with the carbon-to-carbon linkage occurring at the position of the methyl group of acetic acid (Slade and Werkman, *Arch. Biochem.,* 2:97–111, 1943). Although they thought that this occurred by direct condensation of two moles of acetate, it is now known that this conversion occurs by the malate synthetase reaction involving acetyl-COA and glyoxalate.

Werkman and his graduate students had a long-term interest in the physiology of the butyric acid–butyl alcohol bacteria. With the availability of the heavy carbon isotope and the mass spectrometer it was, therefore, a natural consequence that these investigative tools would be employed in the study of these bacteria. In further investigation of the mechanism of the butyl alcohol fermentation (Wood *et al.*, *J. Am. Chem. Soc.*, 66:1812–18, 1944) they stated that "for an understanding of the mechanism of formation of butyl alcohol, it was necessary to know not only the position of the heavy carbon in the carbon chain but also the concentration of heavy carbon in each position. Such information is essential in deciding whether two molecules of acetic acid unite, for example, as follows:

$$2CH_3 \cdot {}^{13}COOH \rightarrow CH_3 \cdot {}^{13}CO \cdot CH_2 \cdot {}^{13}COOH + H_2O$$

$$CH_3 \cdot {}^{13}CO \cdot CH_2 \cdot {}^{13}COOH + 8H \rightarrow CH_3 \cdot {}^{13}CH_2 \cdot CH_2 \cdot {}^{13}CH_2OH + 2H_2O$$

or, whether the acetic acid units with an intermediate compound from the corn starch. In this latter case, probably only one position in the molecule would contain a concentration of heavy carbon in excess of the normal, since that portion of the molecule arising from the starch would have a normal concentration of ^{13}C." In order to determine precisely the location of the heavy carbon atoms, it was necessary to develop a method of degrading butyric acid to achieve selective isolation of fragments of the carbon chain. This was accomplished by a modification of the hydrogen peroxide oxidation procedure of R. H. Allen and E. J. Witzemann (*J. Am. Chem. Soc.*, 63:1922–27, 1941). The oxidation products, which included carbon dioxide, acetic acid, acetone, acetaldehyde, propionaldehyde, and an unidentified non-volatile compound, were determined quantitatively and their ^{13}C contents were measured. The degradation of butyric acid and the determination of the location of ^{13}C atoms in the oxidation products are excellent examples of the technical precision characteristic of Werkman's laboratory.

With the exception of the Booth-Green mill, the work of W. P. Wiggert, Milton Silverman, M. F. Utter, and Werkman (*Iowa State Coll. J. Sci.*, 14:179–86, 1940) was the first to show that extracts could be prepared from bacteria (as had been done with yeast and muscle) which dissimilate carbohydrates. The availability of these enzyme preparations permitted some of the first studies of glycolytic enzymes in bacteria, especially by Utter and Werkman (*J. Bacteriol.*, 41:5, 1940; *J. Bacteriol.*, 42:665–76, 1941; *Biochem. J.*, 36:485–93, 1942). These studies demonstrated that bacteria have fermentative pathways involving many of the same reactions as yeast and muscle.

G. Kalnitsky and Werkman (*J. Bacteriol.*, 44:256–57, 1942, and *Arch. Biochem.*, 2:113–24, 1943) employed a cell-free enzyme preparation obtained by grinding a mass of *Escherichia coli* cells with powdered glass, with subsequent extraction using phosphate buffer (Wiggert and Werkman's method), in the anaerobic dissimilation of pyruvic acid; the result was the formation of acetic, formic, and succinic acids and carbon dioxide, and with a trace of lactic acid. The enzyme preparation contained very active formic dehydrogenase and hydrogenase activity. $^{13}CO_2$ was fixed in formic and succinic acids. The quantity of $^{13}CO_2$ in formic acid suggested that it was formed from the pyruvic acid. The formation of succinic acid from pyruvate and carbon dioxide, with the ^{13}C in the carboxyl group, indicated the fixation of CO_2 with the formation of a carbon-to-carbon linkage.

During his extended career Werkman and his associates investigated a relatively wide range of the biochemical activities of bacteria. It is appropriate to mention briefly some of the other investigations which have not been treated more extensively in this paper. Helen J. Weaver (1927) was perhaps Werkman's first graduate student; she was involved in the study of the bacteriological spoilage of canned vegetables. Shortly thereafter, Gertrude Sunderlin (1928) studied the synthesis of vitamins by microorganisms. Sara Kendall (1931) made a systematic study of the propionic acid bacteria; G. Gillen (1932) studied the production of trimethylene glycol by bacteria; Roger Patrick (1933) studied the xylan-fermenting bacteria; C. A. Johnson and H. D. Coile (1933) devised an electron tube potentiometer for the determination of oxidation–reduction potentials; and Carl Erb (1936) participated in the design of both a multiple-cup micro- and multiple-cup macro-respirometer, which he employed in studying the aerobic dissimilation of lactic acid by the propionic acid bacteria and which were used in many subsequent investigations by Werkman and his students. Milton Silverman (1938, 1939) was among the first to demonstrate the role of vitamin B_1 and cocarboxylase in bacterial metabolism; A. A. Andersen (1940) studied the growth factor and amino acid requirements of bacteria and described a dextro-lactic acid-forming organism of the genus *Bacillus;* Milo N. Mickelson (1940) investigated the mechanism of the dissimilation of glycerol and the formation of trimethylene glycol by organisms related to the coli-aerogenes group of bacteria; and M. E. Nelson (1940) studied the dissimilation of levulose and other substrates in the lactic acid fermentation. Carl Brewer (1939, 1940) investigated the aerobic and anaerobic dissimilation of citric acid by the coliform bacteria, W. S. Waring (1944) the function of iron in microbial metabolism, David Paretsky (1947, 1950) the mechanism for the conversion of 2,3-butylene glycol to acetylmethylcarbinol in bacterial fermentation, and Noel Gross (1947) the isotopic composition of acetylmethylcarbinol produced by yeast juice from ^{13}C-labeled acetaldehyde and pyruvate. A. G. C. White (1947) investigated the assimilation of acetate by yeast and the use of fatty acids in fat synthesis; Samuel Ajl (1948, 1949) studied the mechanism of carbon dioxide replacement by dicarboxylic acids, which by amination, transamination, or similar reactions serve as substitutes for carbon dioxide; and G. E. Wessman (1950) demonstrated the inhibition of carbon dioxide fixation by avidin.

During the period of about 1950 until his death in 1962, it is not unlikely that Werkman found it somewhat difficult to maintain the cohesiveness of his research program and the momentum which his laboratory had experienced in earlier years. His energy and scientific drive were curtailed measurably by a chronic illness which worsened progressively, but nevertheless he was active with his students and in the affairs of the Department of Bacteriology almost to the very end. It is evident from the publications coming from his laboratory that he attempted to continue work on carbon dioxide fixation as well as to develop some new directions. Examples of the research of his students during this period are as follows: dismutative assimilation of carbon dioxide (Dean Watt, 1950–1954), bacterial synthesis of purines (W. B. Sutton, 1951–1953), bacterial synthesis of amino acids (Eric Fowler, 1952) bacterial metabolism of amino acids (Mitchell Korzenovsky, 1953), mechanism of aerobic dissimilation of glucose (C. A. Claridge, 1954), formation of adenosine by cell-free bacterial extracts (John Ott, 1954), the role of transamination in bacterial metabolism (D. H. Hug, 1958), chemoautotrophic fixation of carbon dioxide by bacteria of the genus *Mycobacterium* (T. Myoda, 1960), carbon dioxide fixation by heterotrophic and photosynthetic bacteria (D. S. Bates and C. L. Baugh, 1960), and fatty acid carboxylation by cell-free bacterial extracts (G. W. Claus, 1961–1962).

Seldom does an individual scientist stand alone in his contributions to the body of knowledge; more often than not his reputation and recognition result from the force of his leadership compounded with the efforts of his graduate students and his younger associates in research, and this was certainly true in regard to C. H. Werkman. In any attempt to review his accomplishments as a distinguished American scientist it would be impossible to dissociate his individual work from that of the numerous younger scientists who were associated with him over a period of several decades. This fact is recognized here, and it should be emphasized, moreover, that in a memoir of this nature it is not possible to record specifically the extensive basic contributions to the reputation of the Werkman laboratory which were made by his many students. The impressive bibliography attached to this paper must be viewed as the composite contribution of Werkman and his associates, and the research reports cited specifically should serve only to illustrate some of the directions of the research effort stimulated by Werkman as the leader of his laboratory.

Werkman was a member of the faculty of Iowa State University in the Department of Bacteriology continuously from 1925 until his death in 1962. He served as Assistant Professor, 1925–1927, and Associate Professor, 1927–1933. He attained the rank of Professor in 1933, became department head in 1945, and continued in this capacity until his death. He served as major professor for more than fifty graduate students, of whom thirty-six received the Doctor of Philosophy degree; he was author and co-author of at least 275 publications in scientific journals. For various periods he served as an editor of the following scientific journals: *Archives of Biochemistry, Advances in Enzymology, Proceedings of the Society for Experimental Biology and Medicine, Enzymologia* (assistant editor), *Biotek Publications* (assistant editor), and *Iowa State College Journal of Science.* In 1944 Werkman received the degree of Doctor of Science *honoris causa* from Purdue University, and in 1951 he received the Pasteur Award.

Dr. Werkman was elected to the National Academy of Sciences in 1946; he also held membership in the following organizations: American Society for Microbiology, American Chemical Society, Society of American Biological Chemists, Biochemical Society of Great Britain, American Association for the Advancement of Science (Fellow), Society of Experimental Biology and Medicine, Iowa Academy of Science, Society of the Sigma Xi, Phi Kappa Phi, Phi Lambda Upsilon, and Kappa Delta Pi. In 1958 he was the recipient of the Iowa State University Faculty Citation. He served as a member of the Board of Trustees of the Carver Research Foundation of Tuskegee Institute.

BIBLIOGRAPHY

KEY TO ABBREVIATIONS

Antonie van Leeuwenhoek J. Microbiol. Serol. = Antonie van Leeuwenhoek Journal of Microbiology and Serology
Arch. Biochem. = Archives of Biochemistry
Arch. Biochem. Biophys. = Archives of Biochemistry and Biophysics
Biochem. J. = Biochemical Journal
Ind. Eng. Chem. = Industrial and Engineering Chemistry
Ind. Eng. Chem., Anal. Ed. = Industrial and Engineering Chemistry, Analytical Edition
Iowa Agr. Exp. Sta. Res. Bull. = Iowa Agricultural Experiment Station Research Bulletin
Iowa State Coll. J. Sci. = Iowa State College Journal of Science
J. Agr. Res. = Journal of Agricultural Research
J. Am. Chem. Soc. = Journal of the American Chemical Society
J. Bacteriol. = Journal of Bacteriology
J. Biol. Chem. = Journal of Biological Chemistry
J. Infect. Diseases = Journal of Infectious Diseases
Proc. Iowa Acad. Sci. = Proceedings of the Iowa Academy of Science
Proc. Nat. Acad. Sci. = Proceedings of the National Academy of Sciences
Proc. Soc. Exp. Biol. Med. = Proceedings of the Society for Experimental Biology and Medicine

1921

With William M. Gibbs. Tuberculosis of poultry. Idaho Agricultural Experiment Station Bulletin 126.

1922

With W. M. Gibbs. Effect of tree products on bacteriological activities in soil. I. Ammonification and nitrification. Soil Science, 13:303–22.

1923

Immunologic significance of vitamins. I. Influence of the lack of vitamins on the production of specific agglutinins, precipitins, hemolysins and bacteriolysins in the rat, rabbit and pigeon. J. Infect. Diseases, 32:247–54.

Immunologic significance of vitamins. II. Influence of lack of vitamins on resistance of rat, rabbit and pigeon to bacterial infection. J. Infect. Diseases, 32:255–62.

Immunologic significance of vitamins. III. Influence of the lack of vitamins on the leukocytes and on phagocytosis. J. Infect. Diseases, 32:263–69.

With Max Levine. *Bacterium coli* and *Bacterium aerogenes* in swimming pools. Journal of the American Water Works Association, 10:620–22.

1924

Theory of dye utilization in bacterial media. J. Bacteriol., 8:295–97.

With V. E. Nelson and E. I. Fulmer. Immunologic significance of vitamins. IV. Influence of lack of vitamin C on resistance of the guinea-pig to bacterial infection, on production of specific agglutinins, and an opsonic activity. J. Infect. Diseases, 34:447–53.

With F. M. Baldwin and V. E. Nelson. Immunologic significance of vitamins. V. Resistance of the avitamic albino rat to diphtheria toxin; production of antitoxin and blood pressure effects. J. Infect. Diseases, 35:549–56.

1925

Continuous reproduction of microorganisms. Science, 62:115–16.

1926

The role of accessory food factors in the physiology of microorganisms. J. Bacteriol., 11:86–87.

1927

Microbiological death rates. Proc. Iowa Acad. Sci., 34:85–87.

Vitamin effects in the physiology of microorganisms. J. Bacteriol., 14:335–47.

With H. J. Weaver. Studies in the bacteriology of sulphur stinker spoilage of canned sweet corn. Iowa State Coll. J. Sci., 2:57–67.

With Helen Weaver. Bacterial blackening of canned vegetables. Proc. Iowa Acad. Sci., 34:92–93.

1928

Factors influencing the death time of microorganisms. Proc. Iowa Acad. Sci., 35:97.

With Gertrude Sunderlin. Synthesis of vitamin B by microorganisms. J. Bacteriol., 16:17–33.

1929

Bacteriological studies on sulfid spoilage of canned vegetables. Iowa Agr. Exp. Sta. Res. Bull. 117.

1930

An improved technic for the Voges-Proskauer test. J. Bacteriol., 20:121–25.

Determination of organic acids in mixtures. I. Determination of fatty acids in mixtures by partition between isopropyl ether and water. Ind. Eng. Chem., 2:302–4.

Determination of organic acids. II. Determination of mixtures of two fatty acids by partition between ethyl ether and water. Iowa State Coll. J. Sci., 4:459–64.

Determination of organic acids. III. Note on the use of the isoamyl ether–water system in the partition method. Iowa State Coll. J. Sci., 5:1–3.

With R. H. Carter. Factors influencing the production of acetic acid from corn stalks by thermophilic bacteria. Proc. Iowa Acad. Sci., 37:51–52.

Dimethyl-alpha-naphthylamine for the determination of bacterial reduction of nitrates. Proc. Iowa Acad. Sci., 37:53–55.

With E. I. Fulmer. *An Index to the Chemical Action of Microorganisms on the Non-Nitrogenous Organic Compounds.* Springfield, Charles C. Thomas, Publisher. xiii + 198 pp.

With O. L. Osburn. Determination of butyl and ethyl alcohols in fermentation mixtures. Proc. Soc. Exp. Biol. Med., 28:241–42.

With Roger Patrick. Notes on the bacterial flora of the snake. Proc. Iowa Acad. Sci., 37:57–58.

1931

Determination of organic acids. IV. A method for the provisional identification and quantitative determination of two fatty acids in a mixture. Iowa State Coll. J. Sci., 5:121–25.

With O. L. Osburn. A method for the determination of ethyl and butyl alcohols in fermentation mixtures. J. Bacteriol., 21:20–21.

With E. I. Fulmer and A. L. Williams. The effect of sterilization of media upon their growth promoting properties toward bacteria. J. Bacteriol., 21:299–303.

With E. I. Fulmer and A. L. Williams. Production of bacterial growth stimulants by heating the medium under pressure. Proc. Soc. Exp. Biol. Med., 28:462.

With Sara Kendall. The propionic acid bacteria. I. Classification and nomenclature. Iowa State Coll. J. Sci., 6:17–32.

With O. L. Osburn. Determination of organic acids. V. Application of partition method to quantitative determination of acetic, propionic and butyric acids in mixture. Ind. Eng. Chem., Anal. Ed., 3:264.

With O. L. Osburn. Determination of butyl and ethyl alcohols in mixtures. Ind. Eng. Chem., 3:387–89.

1932

With Charles Davis. Apparatus for continuous fractional vacuum distillation. Chemist-Analyst, 21:20–21.

With G. F. Gillen. Bacteria producing trimethylene glycol. J. Bacteriol., 23:167–82.

With O. L. Osburn. Determination of carbon in fermented liquors. Ind. Eng. Chem., 4:1–6.

With Roger Patrick. A new species of Actinomyces pathogenic in man. Proc. Iowa Acad. Sci., 39:49–51.

With O. L. Osburn. Comparative dissimilation of xylose and glucose by *Escherichia coli* and *Citrobacter anindolcium*. Proc. Iowa Acad. Sci., 39:134–35.

With O. L. Osburn and H. G. Wood. Determination of formic, acetic and propionic acids in fermenting mixtures. Proc. Soc. Exp. Biol. Med., 29:294–95.

1933

With M. C. Brockmann. Determination of 2,3-butylene glycol in fermentations. Ind. Eng. Chem., 5:206–7.

With M. C. Brockmann. Oxidation–reduction studies on the 2,3-butylene glycol–acetylmethylcarbinol system in a fermentation. Proc. Soc. Exp. Biol. Med., 30:1146–48.

With C. S. McCleskey and Howard Reynolds. Physiology of *Shigella paradysenteriae* var. *Sonnei*. Proc. Soc. Exp. Biol. Med., 30:1050–51.

With C. W. Davis and C. A. Tarnutzer. Notes on the trimethylene glycol fermentation. J. Bacteriol., 24:33.

With R. W. Brown. The propionic acid bacteria. II. Classification. J. Bacteriol., 26: 393–417.

With Roger Patrick. Bacterial fermenting xylan. Iowa State Coll. J. Sci., 4:407–18.

With H. Reynolds and O. L. Osburn. Determination of the furfural yielding constituents of plant materials. Iowa State Coll. J. Sci., 4:443–51.

With G. L. Stahly. Physiological studies of the butyl–acetone group of bacteria. I. Gelatinolysis. Iowa State Coll. J. Sci., 7:93–109.

With C. A. Johnson and H. D. Coile. Electron tube potentiometer for the determination of redox potentials. Iowa State Coll. J. Sci., 7:163–75.

With O. L. Osburn and H. G. Wood. Determination of formic, acetic and propionic acids in mixture. Ind. Eng. Chem., Anal. Ed., 5:247–50.

With H. Reynolds and C. S. McCleskey. Sugar dissimilation by *Shigella paradysenteriae* var. *Sonnei*. Proc. Iowa Acad. Sci., 11:81–82.

1934

With H. Reynolds and H. D. Coile. The butyl–acetone fermentation in sugar media. Iowa State Coll. J. Sci., 7:415–26.

With D. B. Charlton and M. E. Nelson. Physiology of *Lactobacillus fructivorans* sp. nov., isolated from spoiled salad dressing. Iowa State Coll. J. Sci., 9:1–12.

With H. Reynolds. Fermentation of artichokes. Proc. Iowa Acad. Sci., 41:75–78.

With H. Reynolds and C. S. McClesky. Dissimilation of sucrose by *Shigella paradysenteriae* var. *Sonnei*. J. Infect. Diseases, 55:207–19.

With G. L. Stahly and O. L. Osburn. Quantitative determination of acetone and ethyl, butyl and isopropyl alcohols in fermentation liquors. Analyst, 59:319–25.

With H. G. Wood. The utilization of agricultural by-products in the production of propionic acid by fermentation. J. Agr. Res., 49:1017–24.

With H. G. Wood. Intermediate products of the propionic acid fermentation. Proc. Soc. Exp. Biol. Med., 31:938–40.

With H. G. Wood. Pyruvic acid in the dissimilation of glucose by the propionic acid bacteria. Biochem. J., 28:745–47.

With H. G. Wood. The propionic acid bacteria. On the mechanism of glucose dissimilation. J. Biol. Chem., 105:63–72.

1935

With B. W. Hammer, G. L. Stahly, and M. B. Michaelian. Reduction of acetylmethylcarbinol and diacetyl to 2,3-butylene glycol