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PERRY WILLIAM WILSON

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A Biographical Memoir by
ROBERT H. BURRIS

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PERRY WILSON, more than any other individual, turned studies of biological nitrogen fixation from a descriptive to a quantitative and analytical emphasis. Nitrogen deficiency more frequently limits plant growth than does any other deficit except water. Certain procaryotic organisms can convert nitrogen from the atmosphere to a form that plants can use. Wilson's research laid the groundwork for the phenomenal increase in studies on the biochemistry, genetics, and physiology of biological nitrogen fixation, a process vital to maintenance of the nitrogen cycle on earth.

Perry William Wilson was born in Bonanza, Arkansas. The family moved from Bonanza to Oklahoma and thence to Terre Haute, Indiana, when Perry still was very young. The possessions and income of the family were modest.

Perry Wilson, in an autobiographical sketch introducing the 1972 *Annual Reviews of Microbiology* about "Training a Microbiologist," said,

My thesis is that one's training comes from many sources, none of which should be overlooked or overemphasized. . . . A widely held belief is that one's career often reflects early influences. . . . As the twig is inclined. . . . My own early training can hardly furnish a test case since I never attended a school long enough to become inclined toward anything. A member of a somewhat mobile family, I attended elementary schools in Arkansas, Oklahoma and Indiana, none for a period of more than two years. . . . The curriculum in such schools was based largely on the three Rs—I became proficient in only two—reading and arithmetic. . . . Biology consisted of courses in human physiology decorously taught from a text suitable for a mixed audience. . . . The text and illustrations were studiously neutral with respect to reproduction. However, such deficiencies mattered not at all; to a student body made up largely of farm children, the facts of life were a part of their daily experience. . . . My favorite course was arithmetic. . . . The problems were oriented toward the practical. . . . In a culture where a mortgage on crops and land was another fact of life, a great deal of attention was given to the calculation of partial payments on bank loans. . . . High school was different. Our family finally settled in Terre Haute, Indiana, and I had the unique experience of attending all four years in an excellent high school. . . . It was staffed by a group of well-trained, young, enthusiastic teachers; they inspired many of us to dream of such a career. . . . In my senior year I took chemistry, but the experience did not alter my plans for a career: attend the local teacher's college and become a professor of high school mathematics.

After completing his high school work in 1920, Perry Wilson received a college scholarship, but it was inadequate to cover expenses, so he took a job as lab boy at the Commercial Solvents Corporation (CSC) in Terre Haute. There had been a great demand during World War I for acetone for use in explosives and in "dope" for airplane wings, and Commercial Solvents had erected a butanol–acetone fermentation plant in Terre Haute. They used Chaim Weizmann's culture of *Clostridium acetobutylicum* which produced butanol, acetone and ethanol in the proportions 60:30:10. Although the demand for acetone slackened after the war, the demand for butanol expanded as a market was created for it in automobile lacquers, so the plant continued to operate.

Perry's first job at CSC was to collect samples periodically from the various tanks being used for production of inoculum for the 40,000-gallon fermentation tanks of corn mash. The distillation of solvents was done from copper pots, and Perry enjoyed keeping them bright. Mr. Bogin, the supervisor, took an interest in Perry, loaned him books, and taught him analytical methods.

After a period in the fermentation lab, Perry's career goals shifted away from teaching high school mathematics, so in the fall of 1922 he enrolled as a chemical engineering student at Rose Polytechnic Institute in Terre Haute. He continued to work weekends at CSC. The butanol fermentations often would become sluggish, and the yield of solvents would drop precipitously; the basis of the difficulty was baffling. Because of their backgrounds in microbiology and fermentation, E. B. Fred and I. L. Baldwin, members of the bacteriology staff at the University of Wisconsin, were invited by CSC to serve as consultants. They uncovered the fact that some of the difficulties with the fermentations arose from contamination with *Lactobacillus leichmannii*. The plant was a remodeled distillery and had a maze of pipes, valves, and dead ends that never were adequately heated by the steam used for sterilization. When the cul-de-sacs were eliminated in a new plant designed by industrial engineers, this problem with contamination was eliminated. However, some fermentations still became sluggish, and it was only later that the source of the problem was defined.

Wilson was offered a position as analytical chemist at the CSC plant, so he dropped out of school for a year to take the job. This gave him a chance to become acquainted with consultants to the company, as he was responsible for analyses of the experimental fermentations. The company sold fermentation residues as an animal feed, and Perry did the nitrogen analyses to establish that the protein content of the material met standards.

Perry studied for another year at Rose Polytechnic Institute and then returned to CSC in the lacquer research department. There he became involved further in analytical organic chemistry. In the fall of 1925 he was told that the company was going to establish some research fellowships at the University of Wisconsin under the direction of Professors Fred and Peterson. This interested him immediately because he had been indoctrinated on Wisconsin by three Wisconsinites on the research staff, and he was well acquainted with Fred and Peterson from their consulting work at the Terre Haute plant. As Perry still was an undergrad, Fred and Peterson did the necessary bending of the fellowship rules so he could complete his undergraduate work at the University of Wisconsin. So he transferred for the second semester in 1926 and was granted fifty credits for his work at Rose Polytechnic Institute. His record shows that he later was permitted to substitute organic chemistry for physiology, physical chemistry for animal husbandry, and math for an agriculture option. His agriculture course record shows that he took bacteriology, agricultural chemistry, agronomy, botany, chemistry, economics, English, German, and veterinary science. It is evident that he designed a program to give himself a solid background in basic sciences.

Although Perry's previous training had included little biology, the course in bacteriology by W. H. Wright captured his imagination, and he wondered how effectively he could integrate his bent for chemistry with a career in bacteriology. The broad treatment of bacteriology in E. B. Fred's soil bacteriology course convinced him that microbiology was broad enough to cover a whole spectrum of interests. His minor was biochemistry (agricultural chemistry at that time), and he took courses from Hart, Steenbock, Peterson, and Tottingham. For research, Wilson was assigned the task of identifying crystals recovered by Elizabeth McCoy from milk that had been fermented by butyric acid organisms. The crystals were calcium citrate, but when they determined the water of crystallization, they found that the literature value was incorrect. So in 1927 Peterson, Wilson, McCoy, and Fred published a paper, Perry's first, in the *Journal of the American Chemical Society* to correct the value.

In the summer, Perry returned to CSC and was assigned to the bacteriological research division. There he worked with D. A. Legg, who had diagnosed that the sluggish fermentations had been caused by a bacteriophage, then described as the d'Herelle phenomenon. A phage-resistant strain solved the difficulties.

Perry received his B.S. in 1928 and submitted an undergraduate thesis project, "Production of Acetylmethyl Carbinol by *Clostridium acetobutylicum.*"

In September 1928, Perry Wilson returned to Madison to initiate his graduate studies. He continued work in bacteriology and agricultural chemistry under the direction of E. B. Fred and W. H. Peterson, and investigated the nitrogen metabolism of *Clostridium acetobutylicum,* the CSC organism. He intended to return to CSC the next year, but his career was altered at this point. In the spring of 1929, the Frasch Foundation, through the American Chemical Society, awarded $40,000 to the departments of Agricultural Chemistry and Bacteriology to do research over a period of five years on the biochemistry of microorganisms. Half the award was for investigating the biochemistry of symbiotic nitrogen fixation. Professors Fred and Peterson asked Perry, when he had finished his M.S. work in the summer, to shift to the Frasch grant and start working on biological nitrogen fixation. This would delay Perry's Ph.D. for a year but would carry an increased stipend. He reasoned that he could finish his Ph.D. and then go back to industrial fermentation, so he accepted. He neglected to take into account how an intriguing research problem and its many ramifications can keep one bound and occupied for life—he never returned to industrial fermentation.

Perry Wilson completed his M.S. degree in August 1929. He married Helen Evelyn Hansel on September 4, and they settled in Madison so Perry could complete his Ph.D. Theirs was a happy marriage. Perry was inherently a nervous and constantly active person, and it was up to Helen to try to keep things on an even keel. As Helen opined later, "Life with Perry was always exciting, sometimes a little hectic, but never dull."

Back in the laboratory in the fall of 1929, Perry started to learn about growing plants, as his research was to focus on fixation in leguminous plants. To keep occupied during this learning period, he wrote a theoretical paper on the energetics of heterotrophic bacteria with W. H. Peterson and published it in *Chemical Reviews*—his first review. Perry had taken an undergrad course with Warren Weaver on probability and had found it a thoroughly exciting experience. He also had taken a stimulating course on the mathematical foundations of statistics from Mark Ingraham, and this had prompted him to read the work of R. A. Fisher and others. He teamed up with Ethel Kullman, who was examining methods for counting the rhizobia, and they published a statistical inquiry into methods for estimating rhizobia.

With the plant methods under reasonable control, Perry Wilson launched into his Ph.D. studies on the relationship between the concentration of carbon dioxide and the fixation of nitrogen by alfalfa and clover. He completed his Ph.D. in 1932 and submitted a thesis on the biochemistry of nitrogen fixation by the legumes. It is apparent that he had lived up to the expectations of E. B. Fred, W. H. Peterson, and I. L. Baldwin, and after his Ph.D. was awarded, the

Bacteriology Department appointed him as an instructor in 1932, followed by advancement to assistant professor in 1934, associate professor in 1938, and professor in 1943.

The Chemistry Department had wanted a course to acquaint their students with biological science. Bacteriology was willing to accommodate, and Perry was assigned to develop a lecture and lab course that was designed primarily for senior chemistry majors. This course, which appeared under various numbers during forty years, was "Perry's baby." It was more demanding than the usual general course in bacteriology and attracted students who wanted a challenge. After some years, it was taken by most seniors in pharmacy as well as chemistry, and it drew many other students with good science backgrounds. It is interesting that Ed Tatum, future Nobel Laureate, early volunteered to aid in the lab to gain teaching experience. From time to time Perry also taught soil microbiology, bacterial physiology, history of bacteriology, and a course in writing scientific reports.

To back up a bit, biological nitrogen fixation had received considerable attention, because the importance of nitrogen as a major fertilizer element for plants had been recognized widely. Mixing a leguminous crop with a non-leguminous crop was practiced as a beneficial operation in the time of the Romans, but the basis of the benefit was not clear. Boussingault in the 1830s performed careful field experiments that convinced him that leguminous plants, such as peas, accumulated considerably more nitrogen than non-leguminous controls, and he suggested that the nitrogen was derived from air. Liebig, who was the leading organic chemist of the day, assailed the findings of Boussingault without bothering to do any experiments to check their validity. This voice of authority from Germany convinced many, but not all; the French continued to support Boussingault. Lawes, Gilbert, and Pugh in England attempted to resolve the issue with very carefully performed experiments, but their careful preparations destroyed the nitrogen-fixing bacteria upon which the legumes depend. Finally, in 1886, Hellriegel and Wilfarth in Germany reported convincing evidence that nodulated leguminous plants can utilize molecular nitrogen. The information was rather quickly reduced to agronomic practice, and in time it became an accepted practice to inoculate leguminous seeds with suitable root nodule bacteria at the time of planting, so that their roots would become properly infected to form nodules. The period 1886 to 1932 was marked by the isolation of root nodule bacteria (*Rhizobium* sp.) in pure culture, demonstration of their specificity for certain leguminous plants, study of the physiology of the organisms, investigation of the infection process, and attempts to get the rhizobia to fix nitrogen apart from the host plant.

The biochemistry of nitrogen fixation was largely neglected. Dean Burk had the idea that this would be a fascinating area of study, so as a postdoc in Meyerhof's lab, he launched studies on the free-living, aerobic nitrogen fixer *Azotobacter chroococcum.* He utilized manometric techniques to investigate the respiration of the organism and attempted to establish its response to changes in the pO_2 and pN_2. Burk stayed with these studies for about a decade and then shifted his research to an even more elusive subject, cancer. Although the constants reported by Burk were not very accurate, he established a new approach for studies of nitrogen fixation.

The unity of biochemistry was being stressed, and Perry was intrigued by the possibilities in developing the comparative biochemistry of nitrogen fixation. He chose the leguminous plant system rather than a free-living nitrogen fixer for investigation. Perhaps this was because the Frasch grant specified work with legumes. The complex symbiotic system is much more difficult to manipulate experimentally than an organisms such as *A. chroococcum,* but as Perry stated on the occasion of receiving the Pasteur Award in his lecture "Chance Favors the Prepared Mind,"

> The first piece of luck arose because we chose the wrong experimental material with which to make the study—the symbiotic system of leguminous plants and the root nodule bacteria. Today we realize that this system is far too complicated for an initial survey and that we should have used species of the free-living soil bacteria, either *Azotobacter* or *Clostridium.* But had we done so, we undoubtedly would have missed the significant observation that gave us the break we needed.

The break to which he referred was the discovery that hydrogen is a specific and competitive inhibitor of nitrogen fixation.

Red clover inoculated with *Rhizobium trifolii* was chosen as the experimental plant. The seeds were surface sterilized, and after being germinated aseptically they were inoculated and transferred to 9-liter Pyrex serum bottles containing sand with plant nutrient minus nitrogen. The cotton stoppers that had been in place for sterilization of the units were replaced with stopper assemblies that allowed evacuation and gas addition through cotton filters. Units were evacuated, and gases were added to desired pressures. An internal indicator showed when it was necessary to add carbon dioxide. Other gases were changed weekly, and plants were grown about six weeks before harvesting.

Although the technique of growing plants in closed containers under controlled gas atmospheres was time-consuming, it yielded interesting results, most of which were summarized by P. W. Wilson in his 1940 monograph "The Biochemistry of Symbiotic Nitrogen Fixation." In approximately a decade, Perry and his research group had defined the growth substance requirements of the rhizobia, studied the respiration of the organisms, investigated and disproved claims that aseptic germinating legume seeds fix nitrogen, investigated the carbohydrate–nitrogen relationship, established the effects of carbon dioxide and light intensity on fixation, checked the claims for excretion of nitrogen from legume roots, established the pN_2 function and the pO_2 function in nitrogen fixation, determined the Michaelis constant for nitrogen fixation in red clover, studied the associated growth of legumes and non-legumes, and reported on the energetics of nitrogen fixation. Perry's monograph summarized this research, and its publication became a milestone in biological nitrogen fixation and a worthy successor to the 1932 monograph of Fred, Baldwin, and McCoy entitled "The Root Nodule Bacteria and Leguminous Plants."

The decade of the thirties included a year when Perry Wilson did research abroad on a Guggenheim fellowship. In his letter supporting Perry's application for the Guggenheim fellowship, E. B. Fred stated, "Perry Wilson . . . possesses an unusual capacity for productive scholarship. . . . He is a clean-cut young man of sterling qualities. . . . In my opinion he is the most promising young man in the field of the biochemistry of microorganisms which we have ever had at Wisconsin."

In 1936, the Wilsons went to Cambridge, England, on the Guggenheim fellowship. There Perry worked with Marjory Stephenson's group to test whether hydrogenase was somehow associated with biological nitrogen fixation. His stay there also provided an opportunity to learn about enzymology and enzymological methods. Perry utilized manometric techniques in his studies, and upon his return to Madison acquired a Warburg respirometer unit for his lab. Cambridge was a hotbed of activity in enzymology, both in the biochemical laborary and in the Molteno Institute where Keilin had his group. Hopkins, Dixon, the Piries, Dorothy Needham, Lwoff, David Green, Baldwin, D. D. Woods, and Perry's colleagues Stare and Baumann from Wisconsin all were there at the time. At the Second International Congress for Microbiology in London, he met Kluyver, Van Niel, Schoen, Stapp, Orla-Jensen, Krebs, and Virtanen. Perry read a paper of his own and one for Dean Burk at the meetings.

At the congress, Perry had an opportunity to discuss his research with A. I. Virtanen. He had not had any success in demonstrating hydrogenase in free-living rhizobia at Cambridge, so he thought he should explore bacteria in the root nodules. Virtanen's Helsinki lab was well equipped for plant work, so he accepted Virtanen's invitation, and he, Helen, and their two-year-old daughter, Gwenn, took a ship to Helsinki in October.

Virtanen had published observations on excretion of nitrogen from leguminous plants. He not only attributed significance to excretion in mixed cropping between legumes and non-legumes, but he reported the recovery of specific compounds among those excreted. The occurrence of aspartic acid among the excreted compounds was a cornerstone in Virtanen's hypothesis that hydroxylamine was the key intermediate in biological nitrogen fixation. Work in Perry's lab and other labs had never shown appreciable excretion, and as a result it had not been possible to verify Virtanen's results or to test his speculations. During his stay in Helsinki Perry didn't find hydrogenase in root nodules, but he did observe a meager level of excretion.

Upon Wilson's return to Madison, the excretion experiments were repeated and were negative. Perry had brought some of Virtanen's peas, barley (non-leguminous indicator plant), and rhizobia for inoculum, and he even had shipped some of Virtanen's sand. None of these helped. George Bond also had no success in getting excretion in Glasgow, Scotland, or in Madison, where he spent a year in Perry's lab. So the controversy on excretion and its significance continued, but Virtanen and Perry always remained on friendly personal terms. As Perry has stated, "Although . . . Virtanen and I disagreed in the public prints regarding the excretion data for the biochemical mechanism of nitrogen fixation, our differences of opinion never affected our warm friendship. We routinely exchanged data before publication, with perfect confidence that no advantage would be taken of the advance information."

When the Frasch grant expired in 1939, the foundation gave another year of support so Perry could write up his results and publish his monograph "The Biochemistry of Symbiotic Nitrogen Fixation." The data were masterfully presented in the monograph, and like the earlier book of Fred, Baldwin, and McCoy, it had an immediate and lasting impact on the field. The scientific literature is now so vast that one can read but a small fraction and assimilate even less. However, a skillfully presented and carefully reasoned treatment such as given in Wilson's monograph still can have a notable influence on a field of research.

After the Frasch grant, Perry's research was supported for years by the Rockefeller Foundation and the Wisconsin Alumni Research Foundation. Emphasis was shifted from leguminous plants to the more easily manipulated free-living nitrogen fixers. The lab group soon established that hydrogen was a specific, competitive inhibitor of nitrogen fixation in *Azotobacter vinelandii* as well as in the red clover studied earlier. *Azotobacter* also proved to have a hydrogenase.

I came to Perry's lab in 1935, took my Ph.D. in 1940, and spent a year during 1940–41 with Harold Urey, Rudolf

Schoenheimer, David Rittenberg, and Sam Trelease at Columbia University absorbing the fundamentals of using the stable isotope ^{15}N as a tracer. Back in Madison, Wahlin and Eppling in the Physics Department helped us build an isotope-ratio mass spectrometer so we could analyze ^{15}N. Perry's group adopted the technique, and we worked jointly until we had accumulated sufficient data to feel confident in supporting ammonia as the key intermediate in nitrogen fixation. The data were far more convincing than those put forward for hydroxylamine by Virtanen, and this marked the demise of hydroxylamine as a candidate for key intermediate and the rise of ammonia to a virtually unchallenged position.

Kamen and Gest in 1949 reported that *Rhodospirillum rubrum*, a photosynthesizing bacterium, fixes nitrogen. They came to Madison to check their observations with ^{15}N, brought active cultures, and within a day we had jointly verified that the organism fixed nitrogen. This was an interesting development, because *R. rubrum* had been a favorite organism for study, but its capability for fixing nitrogen had been missed. It was particularly embarrassing to researchers in the field of nitrogen fixation who had used *R. rubrum* for other types of investigations. Another interesting aspect of the discovery was that Kamen and Gest had been prompted to test the organism, because they had found *R. rubrum* had a hydrogenase, and Perry Wilson for years had been speculating that there must be an association between nitrogen fixation, hydrogenase, and hydrogen inhibition. Perry's group promptly demonstrated that the other types of photosynthetic bacteria fixed nitrogen.

Today, the research on biological nitrogen fixation has expanded greatly, and it is difficult for the work of one individual and his associates to have an impact comparable to that of Perry Wilson's. Perry always had an inquiring mind, and in the thirties he was curious about how the literature on nitrogen fixation had grown. So he investigated this point, and in 1935 he and Fred wrote a paper entitled "The Growth Curve of a Scientific Literature—Nitrogen Fixation by Plants." They found that the number of papers on biological nitrogen fixation had shown a modest growth until the 1886 report of Hellriegel and Wilfarth shifted it into an exponential phase that broke somewhat during World War I. Their extrapolation beyond 1935 was a woeful underestimate, for they stated, "In conclusion, it appears from the 'smoothed' data that the research student of the future can look forward to an annual production of approximately 100 publications a year in this field. This limit of production seems likely to occur about 1965 to 1970. Likewise, the total number of pages to be mastered each year will be from 1,500 to 1,600 before the harassed student may look for relief from an ever-increasing annual load." I counted the nitrogen fixation papers and their pages in the 1965 *Chemical Abstracts,* and in memory of E. B. Fred and P. W. Wilson I will not record the numbers here. Suffice it to say that these giants, who were authors of two of the most definitive books in the field, were highly perceptive individuals but exhibited no psychic insight into how overwhelming our scientific literature would become.

Perry Wilson directed a highly productive research group until the time in 1972 when he had a serious stroke while lecturing to a large bacteriology class. The stroke destroyed his speech and left partial paralysis on his right side. He recovered quite well and led a cheerful life. The tragedy was that the attack that occurred only about a year before his scheduled retirement destroyed his ability to write. Perry was an unusually talented writer. While most of us struggle with transferring thoughts to paper, he appeared to do it effortlessly and with style. He had looked forward to retirement as a time for writing. Fortunately, he had an opportunity earlier as author of his 1940 monograph and his textbook with Werkman on bacterial physiology, as editor of other books, as editor of *Bacteriological Reviews,* and as author of many scientific papers to leave us the benefit of his skills.

Perry Wilson was a good academic citizen and participated in many functions at the University of Wisconsin. He enjoyed teaching and regularly gave a lecture and lab course in bacteriology for advanced chemistry and pharmacy students. He and C. A. Elvehjem led a seminar on respiratory enzymes, and in 1939 the two edited a book entitled *Respiratory Enzymes.* This seminar and book were the genesis of the Enzyme Institute at the University of Wisconsin, and Wilson and Elvehjem were instrumental in its founding. Later Perry did joint research with several investigators at the Enzyme Institute and took leave to work there one year. Wilson served on the National Research Council as representative from the American Society for Microbiology. He was elected to the National Academy of Sciences in 1956 and gained great satisfaction from this. His son recalls that on the day of election Perry left work early and picked him up at West High, and when Richie asked what the excitement was, Perry replied, "Richie, I've been elected an All American."

This comment emphasizes Perry's continuing interest in sports. He was not an athlete himself, but he was always informed about the performances of teams from the University of Wisconsin, the local high schools, and the Green Bay Packers. He vicariously enjoyed the triumphs of his son in cross-country competition.

When Perry was disabled in his later years, he received the loving care of his wife, Helen. They were married in

1929, raised two children, Gwenn and Richard, participated in many activities together, and had a wide circle of friends. Although the years after 1972 were difficult, Perry seemed to enjoy them thoroughly. The absence of earlier responsibilities appeared to give him a lift, and he delighted in seeing people and they enjoyed his company.

Perry Wilson was an unsually fine scientist. He had an excellent background in mathematics and an intuitive grasp of statistics. I have never been associated with a colleague with a better sense of experimental design. His research was well conceived, definitive, and interpreted with honesty and insight. Perry made very few errors in his reported investigations. His submitted papers must have been frustrating to editors looking for something to criticize and correct.

Research support was very limited during the thirties, and Perry Wilson was fortunate to have the Frasch Foundation grant. The combined grant to bacteriology and biochemistry was about $10,000 a year, and those of us who were grad students in Perry's lab were the envy of others in the department because of our affluence. As grad students we received excellent training from Perry, and there was good morale in the lab. Perry was not a hard taskmaster, and we didn't resent that fact that our journal discussion meetings were held in Perry's office at 10:00 a.m. on Sundays.

Perry's talents were recognized by his microbiological colleagues, and he not only served as editor of *Bacteriologal Reviews* but also was elected president of the American Society of Microbiology. It was during the period of his presidency that the organization founded the American Academy of Microbiology. Perry gained particular satisfaction from his role in the NSF-backed project to prepare a set of new high school textbooks in biology, as he always was interested in the development of youth. When he gave the lead lecture in 1969 at a symposium on nitrogen fixation before the Royal Society in London, he wrote Graduate School Dean Bock, "I was flattered to be introduced as the dean of biological nitrogen fixation." But that was how he was regarded in the field, and few would have challenged that characterization.

Perry Wilson had a humble background, but he was highly talented and used his opportunities effectively to establish a special place for himself in the areas of biological nitrogen fixation. He planned his research with imagination and created a solid base upon which he, his students, and current investigators have been able to build an enduring structure.

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