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H. Boyd Woodruff

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CONTENTS

Ann. Rev. Microbiol. 1981. 35:1–28

A SOIL MICROBIOLOGIST'S ODYSSEY

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H. Boyd Woodruff [1]

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CONTENTS

The fifteen introductory chapters previously published in the *Annual Review of Microbiology* have provided valuable insights for preparation of the chapter for the 1981 volume. But such reading leads to conflicting emotions

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—first, great pride in being selected to join the distinguished microbiologists who have served as authors; then dismay at the realization that I am the first in the series associated with an industrial research institution. The themes I shall stress, therefore, are the desirable characteristics of an applied approach to research—the personal satisfactions gained, the accomplishments possible in working towards practical objectives with a group of creative associates, and the opportunities provided to be directly of service to society and to microbiology.

These themes have been stated previously in my presidential address before the Society for Industrial Microbiology and as a participant in symposia to consider the educational needs of applied microbiologists (67).[2] My attitudes were given practical expression in a brief compilation of laboratory exercises for science-oriented students who do not have access to a microbiology laboratory (65). Herein, the themes will not be repeated in redundant fashion, but they should become evident in my recounting of the experiences of a fully satisfied practitioner in the applied field.

One tenet of psychology is that childhood experiences shape the adult. My story will confirm it. My interest in the practical as a scientist can be traced directly to emphasis on the practical in childhood.

Home on the Farm

I was born in Bridgeton, New Jersey, in a farm home, son of a family of South Jersey farmers. Fortunately, formal education was considered a treasured possession by my forebears. But it proved impossible for my father, who at high school age lived miles from schools during a period of homesteading in the state of Washington; and it was limited for my mother because of financial needs of her family. However, with respect to education, there was no doubt of my parents' support. My parents were instrumental in bringing a consolidated school system to our New Jersey farm community to replace a group of single-room schools, and it became responsible for my elementary school education.

Real challenge in schooling came somewhat late in the elementary grades, from an eighth grade teacher who believed not in formalized instruction but in students educating themselves. Our class of 17 was organized into various teams that conducted research and wrote reports with

[2]The appended reference list (see *Literature Cited* section) is presented to illustrate the range of experiences that can await those who select the applied area of science as their specialty. It is obvious that industrial research is not a lonely effort. Academic microbiologists often give credit to their graduate students for providing stimuli for their research accomplishments. The industrial microbiologist does not have graduate students. But he does have associates, also creative, who also stimulate. Note, by actual count, that 73 different co-authors are included in my literature listing.

great emphasis on economic geography. I trace my great interest in travel to the early realization that we in the United States are not the center of the world, that we are in fact just one of the many varied cultures vying for position on the earth.

High school brought exposure to scientific principles. But it was not formal courses in science which satisfied. Physics and biology were boringly taught. Mathematics was the more fascinating topic, each problem a challenge to solve, but its application as a lifetime endeavor not obvious. True excitement came from certain laboratory experiences, first in Freshman agriculture, wherein each student was assigned his own fruit tree on a working farm to be pruned according to principles taught. Each student knew that his action would have a direct economic impact on the farmer-owner of the tree. Later, each student was given his own cockerel to convert by his own hands into a capon, to be raised and sold for profit. At county fairs we took part in judging farm animals in competition with experts. Every activity was a challenge; every success stimulating; every failure disheartening.

Chemistry in my Senior year proved equally fascinating, not in the formal experimentation, which was cut and dried, but in the willingness of the teacher to tolerate, even encourage, personal experimentation, sometimes leading to unexpected smells and disruptions of nearby classes, but all with the objective of creativity. We did not know enough to be academic. We were practical—coating a mirror, generating a source of heat to convert water to working steam, making pigments to dye cloth—all useful objectives but, insofar as we knew, our own discoveries.

Those were Depression days. Farmers had ample to eat but little money to spend. My family's financial worries should have been more obvious to me: long night sessions discussing financial matters, the crisis of an unexpected hospitalization, unpaid fertilizer bills, necessity to move from one share-crop farm to another when unpaid bills became overwhelming. But money means little to a teenager. A magazine route provided spending money, a job at the school cafeteria provided free lunches. Fun could be had in abundance at local swimming holes, Grange parties, marshland fishing, crabbing in salt water ponds, and Sunday school socials.

On the Banks of the Old Raritan

High school graduation brought one face to face with reality. University education was the parents' dream, but how to accomplish it in a state which at that time had no state university, no community colleges? As with my dedicated school teachers, I placed my dependence on others. The scientific advisor of a large farming conglomerate not only offered his aid as a reference but convinced authorities at Rutgers University that free room should be provided to me in exchange for labor. At that time, stemming from the Morrill Act of 1862, only the agriculture and engineering curricula at Rutgers were state supported and eligible for reduced tuition. Even that lowered rate became covered by my winning a scholarship established by a local farmer for a county resident planning to major in some aspect of agriculture. The local county agricultural agent outfitted me with raingear, a family friend provided a new suit and, for the first time, I headed northward in New Jersey toward the New York metropolitan area and its attractions and fascinations. My residence at Rutgers on the banks of the old Raritan began.

Even with free tuition, free room, the challenge of interesting courses, a student must eat. Again, people came to my aid. The director of resident instruction at the Agricultural College, Dr. Frank Helyer (a great friend of the students), solved my problem, as he did for others, by convincing the chairman of the Poultry Department to release ten unused poultry houses for student use. Thus, accompanying me to college were 125 white Leghorn hens, whose eggs provided a source of income via a local egg route and a source of food, together with the inexpensive college farm milk and vegetables and supplemented by canned foods provided in abundance by my family.

The chickens yielded a secondary value—immediate participation in college farm research studies. My poultry house became a rainbow of colors as we applied pigments to determine which of the colored fiberboard wall panels the chickens were least likely to peck at and destroy.

I learned quickly that not all experiments lead to desirable ends. After studying in biochemistry about vitamins, I immediately collected and fed my chickens grass as a source of vitamin A, with disastrous effect! My customers refused my eggs with their brilliant yellow yolks. I discovered that New York area housewives want white-shelled eggs with pale interiors.

University professors, too numerous to mention, provided challenging experiences. No more boring science courses! Geology, zoology, botany, chemistry in all its aspects, even agronomy, plant physiology, soil pedology were fascinating. The same could not be said for educational psychology and economics, but one must learn to accept the uninteresting while appreciating the good.

As a soil chemistry major (the usual ploy at that time to obtain a chemist's education at the lower tuition rates of the state-supported agricultural school), my hours were filled with science, but advisors insisted on sufficient liberal arts courses to insure Phi Beta Kappa election, which ensued. After the easy successes possible at my relatively small school, it took a failure to make me recognize my deficiences. As the Rutgers University nominee for a Rhodes Scholarship, I failed the interview sessions miserably because I had no plan for the future, did not know what I wanted to do with my life, and did not understand my need for future supplemental education.

College experiences were wonderful, overwhelming, broadening. I met interesting people, attended concerts for the first time, even tried out for track (at which I came in last at every meet but one). I celebrated through the night when Rutgers (which had started the sport of football by defeating Princeton University in 1869) after 69 years of trying won its second game from Princeton by the exciting score of 29 to 27. At Rutgers I met Jeanette Whitner, the Women's College student who eventually became my wife, the mother of our children, and co-worker in our joint household and career.

My first real contact with industrial research occurred early during the undergraduate period when I was appointed as the Danforth Fellow for my state. Arriving in St. Louis, one representative from each state in the continental US, we entered into a comprehensive training program in business practice at the Ralston Purina Company. This was followed by a period at the company's research farm during which we aided in the conduct of ongoing research projects. My choice, dictated by experience, was poultry science. For the first time, I was exposed to the significance of fully controlled experimentation, a valuable lesson and an introduction to the many double-blind clinical trials I was to experience later during the evaluation of antibiotics.

Then, a turning point occurred—a course in soil microbiology taught by Professor Selman A. Waksman, with laboratory instruction by visiting instructor Wayne Umbreit. Thus, I simultaneously enjoyed two experiences that had a lasting influence on my life: frequent contact with a truly inspirational teacher, and my first introduction to a representative of the University of Wisconsin School of Bacteriology, which has played such a dominant role in the development of my particular area of microbiological interest. For the first time I realized the unity of biology and chemistry, that each biological observation has an underlying chemical cause, that in unraveling the latter, one could understand the other.

Graduate Studies in Soil Microbiology

Graduation from Rutgers in 1939 brought me to the first great decision point in my life, the choice of a graduate career. All preparation had been for chemistry. Letters were written soliciting graduate school appointments. I was not prepared for, and did not expect, an offer received from Dr. Waksman to join his student group on a University fellowship permitting full-time research at the then unusual stipend of $900 a year, fully 20 percent more than fellowships available elsewhere, without the need to perform time-requiring teaching assistantship duties. Still the decision was difficult. Should I stay at Rutgers? Should I leave chemistry for biology? Dr. Jacob Lipman, dean of the Agricultural School, the directors of various departments were consulted, and all pointed to Dr. Waksman's chemical approach to microbiology. Had he not been author of a book entitled *Enzymes*? It was recommended that I examine his publications to see the extent to which chemistry was utilized. I was convinced and decided to stay. The die was cast.

With the enormous financial support for research presently enjoyed by American universities, and the well equipped and staffed laboratories, it is difficult to remember the privations existing in the 1940s. It may be difficult also to recognize my good fortune at the time in joining the soil microbiology laboratory at Rutgers. Dr. Waksman was able to spend half of each day working in the laboratory. As his laboratory assistant I learned directly by example and by discussion. The other member of the departmental staff was Dr. Robert Starkey. He also worked in the same laboratory. His influence, the enthusiasm with which he attacked each research endeavor, his willingness to spend hours in explanation, to teach on a one-to-one basis, the effort he gave to planning group experimentation, all must be considered as having major influence on my development as a scientist.

I deserved the envy of my associates. Who else could report that their entire graduate study program was spent working at the same laboratory bench with two senior scientists, one a future Nobel Prize winner, and both later to serve terms as President of the American Society for Microbiology?

Dr. Waksman, already a member of the National Academy of Sciences, had achieved fame in several fields: soil microbiology, actinomycete taxonomy, enzymology, and marine microbiology. As a result, his department was international in character. I joined with students from mainland China, India, South America, Europe, and various states of the United States. Topics of research were equally varied. Fumaric and citric acid production by molds, nitrogen fixation by azotobacters, the biochemical processes leading to the formation of peat, the effects of microbes and their polymeric products on soil erosion, decomposition of cyanide in soil, nature of marine microorganisms, taxonomy of actinomycetes, associative effects of microorganisms in soil, microbial activity in composts at elevated temperatures, the composting of human feces, the oxidation of sulfur in soil, fungi which grow under conditions of extreme acidity, all were in progress in the soil microbiology department. Results were discussed in depth in seminars. It was all a learning experience. How different from the specialization characteristic of graduate study today!

Antibiotics

Again, a turning point developed. After my brief probes at defining the steps of sulfur oxidation in the soil and applying procedures of proximate analysis to define the changes occurring in composts maintained at 50 and 70°C, Dr. Waksman appeared in the laboratory one day. He was highly agitated. "Woodruff," he said, "drop everything. See what these Englishmen have discovered a mold can do. I know the actinomycetes will do better!" He had just learned of the extension of Fleming's work on penicillin in Florey's Oxford University laboratory, proving its chemotherapeutic potential. Thus was initiated the first search in Waksman's laboratory for antibiotics from actinomycetes.

The work was quickly successful (48, 49, 53). At first, experience was gained in reisolating the old bacterial product pyocyanase, but soon we had actinomycin, a new product to study and evaluate. Fortunately, actinomycin was easy to produce and extract, was sufficiently stable to be studied chemically, and was so highly active that we could dream of practical applications. But all our dreams were defeated by toxicity (46). It was not until much later that the usefulness of the actinomycins in treatment of rare forms of cancer was recognized.

Actinomycin did, however, provide my first introduction to Merck & Co., Inc., the place of my eventual employment. Merck microbiologist Dr. Jack Stokes, a former Waksman student, offered to provide facilities for large-scale production of actinomycin. The hundreds of flasks incubated at Merck were inoculated directly by Waksman himself. I served as assistant and general handyman. Merck chemist Dr. Max Tishler, later to become president of the Merck Sharp & Dohme Research Laboratories, joined with Dr. Waksman in bringing actinomycin to the final state of purity and investigating its structure.

As a result of the early successes, incoming graduate students were assigned to antibiotic research. Many were engaged in screening. A culture isolated by Dr. Walter Kocholaty was assigned to me for further research and from it was isolated streptothricin (51, 54), the first of the basic water soluble antibiotics.

No facilities for animal study existed in the soil microbiology department, but they were available in the dairy department under Dr. H. J. Metzger, who was studying contagious abortion, caused by *Brucella abortus,* in laboratory animals. The disease still was a serious human infection at that time. Our streptothricin concentrate was tested for toxicity and proven safe and then was shown to produce significant cure of experimental infections.

The excitement in our group was intense. From that time on Dr. Waksman's deparment specialized in antibiotics. The advantage of variety in research experience was lost to its students. We talked of antibacterial substances, nothing else (45, 50). There was much debate whether the substances were natural soil products or products of artificial cultural conditions (47, 52). At one lunchtime discussion, Dr. Waksman coined the word *antibiotic* and stated its definition. The word entered the scientific vocabulary.

Microbiology at Merck & Co., Inc.

Wartime had come to the United States. After being refused in his attempt to enlist, Dr. Waksman turned full attention to antibiotics as a cure for disease. During his consultation visits at Merck & Co., Inc., Waksman gave encouragement to the budding interest of the company in microbial processes. Merck was well known for its success in isolation of natural products in a pure state and their chemical synthesis, especially the vitamins. But the time was ripe for change. Merck was already employing microbes in two of its minor manufacturing processes: yeast to convert benzaldehyde to L-phenyl-1-acetylcarbinol, a step in the manufacture of L-ephedrine; and a bacterium to oxidize sorbitol to sorbose, the initial step in production of vitamin C. Biochemist Dr. A. Walti constantly brought new microbial processes to the attention of Dr. Randolph T. Major, the research director. An assistant director, Mr. John C. Woodruff, newly arrived from Commercial Solvents, was convinced the future of the pharmaceutical industry lay with biological manufacturing processes.

Waksman proposed that citric acid and fumaric acid, both projects of his graduate students, were ready for commercial development and convinced Merck & Co., Inc. to build a pilot plant for citric acid production using the new fungus *Aspergillus wentii.* It was an important step because it brought Dr. Lloyd C. McDaniel from the University of Wisconsin to Merck to operate the pilot plant and served to teach the Merck laboratory staff the microbiological techniques that later were to prove vital to success in antibiotic manufacture.

Waksman kept the Merck research directors informed of his work on antibiotics from streptomycetes and strongly suggested the initiation of developmental studies at Merck with tyrothricin, the antibiotic discovery of his former student Dr. Rene Dubos, then working at the Rockefeller Institute in nearby Princeton. But most important, Waksman supported the Merck staff's interest in the Oxford studies on penicillin and convinced Merck to recruit Dr. Jackson W. Foster, (a former assistant of Waksman at Rutgers and recently returned from postdoctorate experience with Dr. C. B. van Niel) as laboratory director for its antibiotic studies.

Penicillin

Merck & Co., Inc. was fully prepared to give favorable reception to Drs. Howard Florey and Norman Heatley when they arrived during the darkest days of the war to request aid of American industry in the production of penicillin. The story of that request and its result has been described in full