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Friend of the Good Earth: [Dr. Renel Dubos]

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Gramicidin Crystals

THE ROCKEFELLER UNIVERSITY

RESEARCH PROFILES

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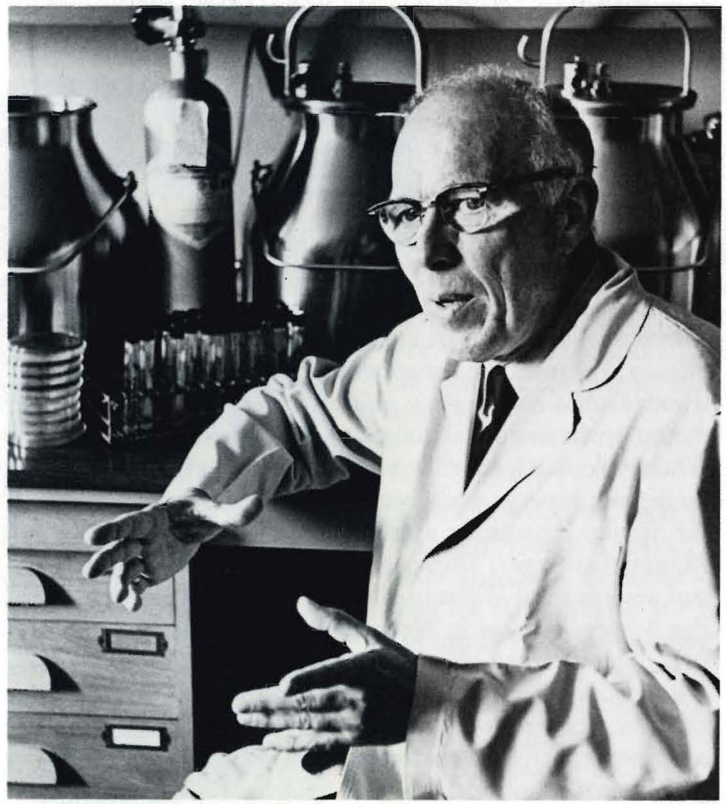
Friend of the Good Earth

Fifty years ago, microbiologist René Dubos taught the world the principles of finding and producing antibiotics. His discovery of gramicidin in 1939, at The Rockefeller Institute for Medical Research, represents the first systematic research and development of an antibiotic, from its isolation and purification to an analysis of how it cures disease. Gramicidin and its less pure form tyrothricin were the first antibiotics to be produced commercially and used clinically. They formed the cornerstone in the antibiotic arsenal and remain in use today.



René Dubos (1901-1982)

This remarkable achievement was not Dubos's first, last, or even his most important contribution. To René Dubos, a living organism—microbe, man, society, or earth—could be understood only in the context of the relationships it forms with everything else. This ecologic view led him from investigating problems of soil microbes to those of specific infectious diseases, to social aspects of disease, and, finally, to large environmental issues affecting the whole earth. His 55-year evolution from microbiologist to environmentalist reflects his efforts to build a new philosophy of man's life on earth.

CHANCE EVENTS AND A PREPARED MIND

As a child, Dubos lived in small French villages north of Paris where his parents ran a butcher shop. He suffered from rheumatic fever and severe myopia that instilled in him a fear of blindness and a shortened life. Frequently confined to bed, he read avidly

Dubos (left) with his family, circa 1908, at his home in Hénonville, France.

Dubos as a student at Collège Chaptal, circa 1914.

and found early heroes in French translations of Buffalo Bill westerns and Nick Carter detective stories. Dubos was inspired to study history when he encountered an essay describing the influences of the Ile-de-France environment on La Fontaine and his fables. These plans were dismissed when his father died in 1919. His mother needed help in the shop, and he suffered another bout of rheumatic fever, preventing him from taking the entrance exam for the Ecole de Physique et Chimie.

On recovering, Dubos passed the exam to the one school still open for enrollment that year. So it was by chance that he attended the Institut National Agronomique to study agricultural science. He excelled in all courses except microbiology. He disliked chemistry and told his mother he would never again enter a laboratory.

After graduation, while writing abstracts for an agricultural journal, Dubos happened to read an article by Serge Winogradsky, a renowned soil bacteriologist. Winogradsky stated that microbes should not be studied in laboratory cultures, but in their natural habitats where environmental conditions and other organisms influence their activities. Dubos embraced this ecological approach to science and decided to study microbiology.

Before resuming his studies, Dubos wanted to visit America. By 1924 he had earned money for passage to the United States on the steamship *Rochambeau*. On board, fate intervened when he met Selman Waksman, the American soil bacteriologist whom Dubos had recently guided around Rome during an international congress on soil science. When Waksman learned Dubos had ambitions but no definite plans to study bacteriology, he offered the young Frenchman a fellowship to study at Rutgers University. Dubos arrived in New York and accompanied Waksman that same evening to the Rutgers campus in New Jersey. Three years later, Dubos earned his doctorate. In the spirit of Winogradsky, Dubos showed that the environmental characteristics of the soil determine which microbes are activated to decompose cellulose, the main ingredient of wood.

This vial of polysaccharide, labeled in Professor Avery's handwriting, enticed Dubos into medical research.

In 1927 two more chance events brought Dubos to The Rockefeller Institute (renamed The Rockefeller University in 1965). His application for a National Research Council fellowship was denied because he was not a citizen. The rejection letter contained a handwritten note suggesting he consult with fellow Frenchman Alexis Carrel at the Rockefeller. Carrel was kind and considerate but could offer no advice. At lunch, in the now legendary dining room in Welch Hall, Carrel sat the visiting Dubos next to Oswald Avery, whose research expertise with the pneumococcus would lead him to the discovery of DNA as the material of heredity in 1944.

A PLAN, SOME PATIENCE, AND A FEW POTS OF SOIL

The origin of antibiotics began in Oswald Avery's laboratory at The Rockefeller Institute Hospital, which was trying to produce a therapeutic serum to cure the deadly disease lobar pneumonia. Avery had established that a polysaccharide capsule, or "sugar coat," surrounding the virulent type III pneumococcus bacteria protected it from the body's defense mechanisms. He spent many frustrating years trying to safely decompose this microbe's sugar coat. He knew whoever could find a way to selectively destroy it without side effects would discover a cure for pneumonia. He needed someone in his laboratory to elaborate on what he called his "kitchen chemistry."

Oswald brought Dubos back to his laboratory after lunch. He waved a vial of the purified polysaccharide and portrayed the scientific drama that lay within. Dubos replied, rather brashly for a new Ph.D., "Well, I think I can find a microbe to decompose that capsule, and from it I can extract an enzyme." Avery, impressed with this promise, arranged a fellowship for Dubos, little realizing that the problem of the pneumococcus capsules would launch a career that would concern itself someday with the atmospheric capsule enveloping the earth.

In 1975 Dubos recalled his acceptance by Avery. "I don't think there was any other institution in the world then—and perhaps there is none now—that would have taken a person like me, knowing nothing at all about medicine, and coming from an agricultural experiment station, and given him a chance to work in a hospital. It has sensitized me, and ever since I have preached that scientific institutions must remain very flexible and very open."

Within three years, Dubos fulfilled the promise he made to Avery. As a soil microbiologist, he knew organic matter did not accumulate in nature, because countless microbes perform limited, well-defined tasks to recycle this matter. He felt it was a matter of time and patience before he found one specialized in removing the capsule from the type III pneumococcus.

Dubos described the utilitarian 1930s laboratory where he cultivated endless pots of soil in a systematic search for the elusive destroyer. He worked at a wooden desk in a large room that "accommodated a motley assortment of notebooks and simple laboratory instruments—test tube racks, glass Mason jars, droppers for various dyes and chemical reagents, tin cans holding pipettes and platinum loops....The Bunsen burner on each desk served for aseptic transfer of cultures, heat sterilization, preparation of culture media, and also for some chemical operations. We used a great variety of kitchen utensils....(The room had) a few simple incubators, vacuum pumps, and centrifuges...(and) a single porcelain sink that served for almost any operation requiring the use of water, from staining slides for microscopic work to preparing extracts of bacterial cultures for immunological tests."

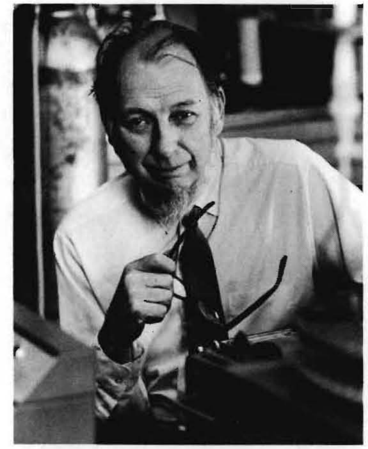
Using a few gardening techniques, he cultivated a soil bacterium from a New Jersey cranberry bog that removed the capsule from the pneumococcus. And, from this bacterium, he isolated an enzyme called the SIII enzyme responsible for the destruction. The triumph came when this enzyme was given to mice infected with pneumonia and it cured them all. Avery was so impressed that he interrupted his summer vacation to verify and publish these findings. The enzyme might have been purified into a therapeutic serum to treat pneumonia in humans, but the sulfa drugs that were just becoming available eclipsed the enzyme's further development. However, Dubos's scientific methods opened the way to gramicidin and the beginning of antibiotics.

A peculiarity of the cranberry bog microbe was that it did not produce the enzyme of its own accord. Dubos discovered the enzyme was produced if the polysaccharide capsule was its only source of food. Once again, he showed the importance of the environment, in this case the soil, in determining which of an organism's multiple potentialities would be manifested. He called this phenomenon "adaptive enzyme," now known as induced enzyme, because enzyme production is an adaptive response to the compelling force of the environment. Dubos considered this his "greatest hour in science," and said it was "one of the most important biological laws I have ever been in contact with."

Dubos had several other successes in finding soil microbes to solve specific biomedical problems. When he decided to look for a soil microbe that could exert broader effects, he used the same cultivation techniques he developed to find the SIII enzyme. This time, however, he tended his soil samples for two years. He wanted to ensure that the only microbe that thrived was one with a cannibalistic appetite for the disease-causing bacteria he was providing as the sole food source to his soil samples. In 1939 Dubos isolated and identified *Bacillus brevis* as the microbe that digested and destroyed other microbes, particularly the pneumococci, staphylococci, and streptococci. From *Bacillus brevis* he extracted the antibacterial agent, or antibiotic, that he named tyrothricin. It contains two polypeptides he called gramicidin and tyrocidine.

Tyrothricin is the partially purified antibiotic whose active ingredient is gramicidin; tyrocidine is ineffective in the body. Within a few months, Dubos and organic chemist Rollin Hotchkiss, a colleague at the Institute, described the bacterial, pharmaceutical, chemical, and clinical aspects of tyrothricin. Although gramicidin proved too toxic to be taken internally, it is highly effective in treating human wounds and other skin infections. Elsie, the famous Borden cow, was stricken with mastitis at the 1939 World's Fair, and was one of the first patients to respond successfully to gramicidin.

Almost immediately, every pharmaceutical company began producing these two antibiotics. Rollin Hotchkiss recalls their 1940 patent application to prevent any restrictions on their production and to ensure their purity: "After an almost hilarious series of negotiations between the unworldly scientists and the worldly lawyers, in which the contents of our notebooks had been translated into thirty-six patent claims, we were called to the Business Manager's office to assign the patent to the Institute.



Professor Rollin D. Hotchkiss, who collaborated with Dr. Dubos to purify and produce the first antibiotic, gramicidin.

When we read the clause that ran 'acknowledging the receipt of the sum of one dollar,' we asked if they were serious about the dollar. The business manager, Edric Smith, mumbled something and withdrew, returning a bit later, presumably from the Bursar's Office, carefully shepherding into the room two half dollars; one for each of us! Dubos, who knew how to make a grand gesture when the time was right, was positively delighted with the evident embarrassment of all concerned." Institute Director Herbert Gasser and Hospital Director Thomas Rivers enthusiastically supported the antibiotic discovery and soon provided the two scientists with new laboratories. The Institute abandoned the patent application in 1943, stating that its objective, to make the discovery freely available to the public, had been achieved.

The work on gramicidin encouraged English scientists Howard Florey and Ernst Chain to revive the dormant research on penicillin that Alexander Fleming found accidentally in 1928. Their article on penicillin's use as a drug appeared in 1940, a year after Dubos's reports on gramicidin. Other scientists began to probe the soil for bacteria that would produce more antibiotics. Dubos's teacher, Selman Waksman, who undertook a search that led to streptomycin, acknowledged that "that gold rush [for antibiotics] should be traced to Dubos's isolation of gramicidin.... To obtain the desired results required an analytical mind, an original coordination of all the facts, and especially a new philosophy...it was the beginning of an epoch."

UPDATING THE GERM THEORY

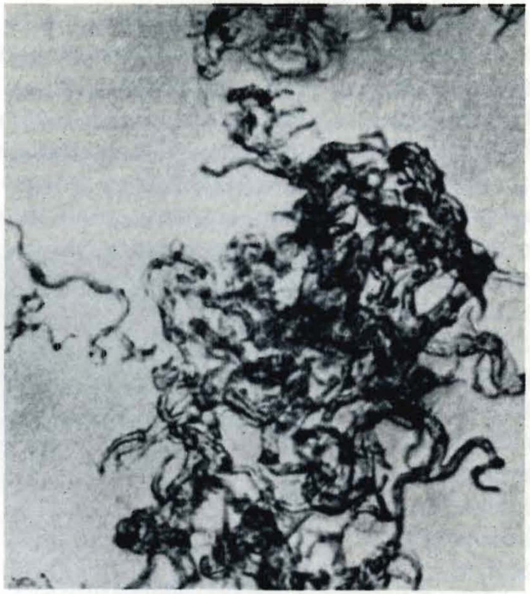
Even before any of the other antibiotics became available, Dubos predicted bacteria would adapt themselves to these drugs and produce resistant strains. While he recognized great victories in the battle against fatal infections, he warned that these drugs could control, but never conquer, their enemies. In his book, *Mirage of Health*, Dubos compared a "conquer mentality" to the cowboy philosophy in a Buffalo Bill western. "In the crime-ridden frontier town the hero, singlehanded, blasts out the desperadoes who were running rampant through the settlement. The story ends on a happy note because it appears that peace has been restored. But the death of the villains does not solve the fundamental problem. The rotten social conditions which had opened the town to the desperadoes will soon allow others to come in, unless something is done to correct the primary source of trouble."

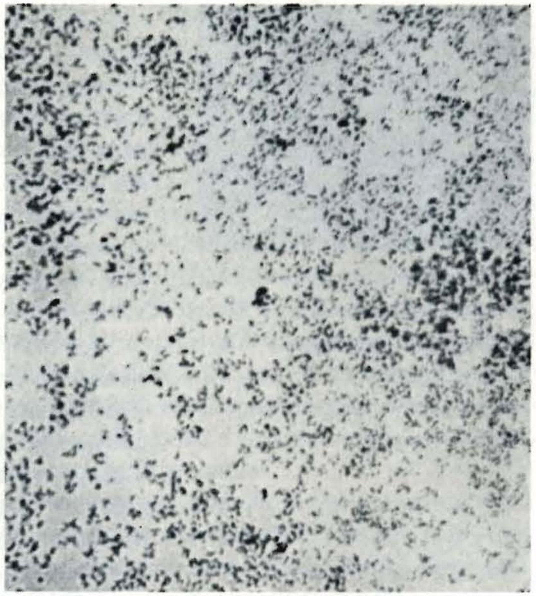
Dubos predicted that increasingly crowded, uniform societies would bring new diseases into being. Eminent scientist, physician, and author Lewis Thomas adds, "Dubos was quite certain that antibiotics in whatever abundance were not going to be the solution. He had a prescient mind and he was especially afraid of what the new viruses might do."

A tragic event in Dubos's personal life turned his interests to the human condition in disease. His first wife died of tuberculosis in 1942, just after he accepted a professorship at Harvard Medical School. Noting that his wife had tuberculosis as a child, he believed it was reactivated by her anguish over family problems in France resulting from the war. Her disease alerted him to the balance between man and bacteria and to the environment's effect on that balance.

After completing wartime studies on dysentery at Harvard, Dubos returned to The Rockefeller Institute in 1944, where he was given complete freedom to establish a laboratory devoted to tuberculosis.

Along with Bernard Davis, Dubos created a culture medium,





Growth of two cultures of tubercle bacilli. Above (left), cells grow in large clumps in a conventional medium. Above (right), cells show well-dispersed growth in the Dubos-Davis medium. The introduction of nontoxic detergents in the culture medium enabled the first accurate, quantitative studies of various strains of tubercle bacilli and of their disease-causing properties.

Dubos flanked by James G. Hirsch (left) and Zanvil A. Cohn (right) at Dubos's 80th birthday party

or special environment, that produces rapid, luxuriant, and well-dispersed growth of tubercle bacilli in the test tube. This advance brought a renaissance in tuberculosis laboratory research. There were also fruitful collaborations with two assistants who returned with him from Harvard. With Cynthia Pierce-Chase, Dubos pioneered methods for the worldwide standardization of BCG vaccination against tuberculosis, thereby acting on his belief that prevention is better than cure. With Jean Porter, who became his wife in 1946, he co-authored *The White Plague,* a definitive history of tuberculosis as a social disease.

In the 1950s, he and the late James G. Hirsch conducted clinical studies on tuberculosis patients in The Rockefeller Institute Hospital. This group determined that prolonged bed rest was not needed and led to the closing of tuberculosis sanitariums, ending an era in medical history. Hirsch recalled how Dubos loved to "play doctor," as he replaced his tan lab coat with a physician's white one and "joined rounds on the ward to visit and show human concern for 'his' patients." This clinical work led to the collaboration of Hirsch and Professor Zanvil A. Cohn, who pursued studies at The Rockefeller University on human defense mechanisms to fight infection and disease. Today, Dr. Cohn continues the tradition of the Dubos laboratory by studying the natural reactions of the human body, collectively known as the immune response, that determine the course and outcome of an infection.

The dining room in Welch Hall in the mid-50s. Dubos said, "The dining room where I first met Dr. Avery was the greatest educational institution I have known anywhere. I came to the [Rockefeller] Institute not knowing a word about medicine. But everyday in the dining room at lunch I became slowly sensitized....My suspicion is that if it had not been for the dining room at the Rockefeller I would not have been as rapidly successful in science."

Lewis Thomas observes that "although Dubos was not a doctor, he learned more about medicine than most physicians. He knew the power of scientific medicine to reverse mortal infections. But he also knew that mankind's changing of his own environment has much more to do with susceptibility or resistance to infection than anything in the modern pharmacopoeia."

In the 1960s, Dubos investigated the effects of malnutrition, toxins, and stress, and demonstrated how these external factors increased susceptibility to tuberculosis. After The Rockefeller Institute became The Rockefeller University, Dubos's graduate students enlarged his investigations by testing modern environmental influences such as crowding, pesticides, and enzymes in detergents on resistance to other diseases.

Working and thinking ecologically, Dubos's revisions in the germ theory implicated the total environment as a determinant of disease. He showed that a microbe is necessary but not sufficient to cause disease. He reasoned that men coexist with microbes, both good and bad. He found disease-producing microbes are not inherently destructive and can persist in a quiescent state in the body for long periods. The important element in disease, he determined, is not infection but rather any stress that alters resistance, provokes the onslaught of illness, and then determines the outcome of the disease. Dubos's new theme became "if we want to improve our physical and spiritual well-being, we must first understand and then control our impact on our surroundings." For these contributions, infectious disease specialist Walsh McDermott referred to Dubos as "the conscience of modern medicine."

THE CONSCIENCE OF HUMAN ECOLOGY

To many, Dubos is known as an environmentalist, a scholarly elder statesman, author of some two dozen books, and spokesman for the health of the earth. He saw this new role as an opportunity to address urgent environmental problems that were anticipated by his unfinished laboratory studies. For him, it was the next step in fulfilling the University motto to put science at the service of mankind.

The transition to environmentalist began in the 1950s. As invitations to lecture on environmental aspects of health and disease grew more numerous, he wrote to University President Detlev Bronk explaining his frequent absences from the campus. He expressed profound gratitude for the opportunity and freedom the University had always provided so that he could develop the cultural as well as the scientific aspects of medicine. Dr. Bronk's reply encouraged Dubos to continue his "double life in the laboratory and on the frontiers of a changing society." When

Dr. Dubos walking to work under the allées *on The Rockefeller University campus.*

Dubos's book *So Human an Animal* won the Pulitzer Prize in 1969, he was drawn into the mainstream of the environmental crusade. With amazing resilience and energy between his 70th and 81st years, he devoted all his skills as speaker and author, coupled with his stature as a scientist, to formulate emerging environmental and social issues for an extensive public audience.

Dubos was a visible environmentalist with his charming French accent and avuncular manners. Tall, vigorous, rosy- cheeked, with durable white wisps on a balding head, he radiated a special *joie de vivre.* One was drawn in immediately by his attentive blue eyes filtered through thick glasses, a shy yet broad smile, and beautiful large hands that enthusiastically punctuated every sentence. He coined numerous mottoes to encompass complex issues, among them "Think globally, act locally," which remains a frequently quoted credo of environmental activists.

Dubos changed the ways we think about the environment. He restated ecology as a science that included man and concerned itself with how people live their lives. William Reilly, then president of The Conservation Foundation, and now Environmental Protection Agency Administrator, said "Dubos's career began with a critique of environmental abuses and their effects and it concluded with a plea for confident and informed intervention in nature, a belief—heretical in conservation—that 'man can improve upon nature'....Dubos stood for creation."

Not surprisingly, Dubos's favorite landscape represents man's enhancement of nature. He said that walking under the *allées* of trees lining the entrance and the marble walks to the University was an important element of his life. "Morning and evening, summer and winter, walking back and forth, I give thanks to those who planted on the grounds of The Rockefeller Institute the rows of sycamore trees which today look so noble against the background of New York City. Always I have in mind the avenue of venerable trees along the roads of France and in the parks where I played as a child." While walking beneath the trees which he watched grow for over half a century, he came to view them as a symbol of the contemplative, peaceful seclusion at The Rockefeller University. "There is no place in the world where I have spent as much time and where I feel more at ease. Whenever I approach the stalwart plane trees of the 66th Street entrance, I know 'this is the place.' " □

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