Rene Dubos and the Emerging Science of Human Microbial Ecology

By Funke Sangodeyi

Ph.D. Candidate, History of Science Department
Harvard University
Cambridge, Massachusetts

funkesan@gmail.com

The story of microbes in historical scholarship and in the history of medical research often starts with a discussion of the germ theory of disease and ends with a discussion of antibiotics, or its consequences (antibiotic-resistant bacteria, for example.) The focus of this work is on infectious pathogenic bacteria. However, the vast majority of bacteria that are implicated in the human body are not disease-causing pathogens. In fact, the body is teeming with these bacteria—there are trillions—orders of magnitude that show more bacterial cells than human cells. Recently, these nonpathogenic bacteria have achieved a kind of celebrity: the National Institutes of Health began a well-covered large-scale inter-institute initiative to study this collection of microbial fellow travelers—historically called the normal bacterial flora—in 2007.[1] This initiative, called the Human Microbiome Project, aims to characterize and begin to better understand the role of the bacterial inhabitants of the body, largely through new genomic techniques.[2] The key technique, metagenomics, takes the genetic material of an environment as its analytical target instead of a sole organism. Instead of mapping the genome of a particular organism, the goal is to map the genetic diversity of a habitat.[3] In the case of the Human Microbiome Project, that habitat is the human body.

Microbiome researchers have taken this idea—that the human body is a microbial habitat to be mapped—and built upon it a new conceptualization of the human body to frame their

findings. In this framing, the human body is more than just a habitat for biologically diverse communities of microbes. Like in any environmental habitat, the habitat is not just background, and the biological activities occurring in it, among other living things, are not just background noise. They are implicated in each other's lives. It is the same with the host-microbe relations as well, these researchers argue. The human body, they tell us, is an ecosystem. Its physiological, immunological, and metabolic functions are implicated in the everyday activities of the microbial communities that are nestled in the various ecological niches that comprise much of the human body.[4] Hence these researchers are claiming an utterly new way of thinking about health, human biology, disease and the body—on both technical and philosophical terms.

This project is not just a genomics project, based on the newer knowledge of genetics and cutting edge computational techniques. It is a project that depends in part on special knowledge of the particular body sites that have been redefined as habitats for microbes. This redefinition has a history—the articulation of the microbe-body relationship was built up piecemeal, often according to body site. My dissertation, entitled, *The Body as Ecosystem: Good Germs and American Bodies,* traces and contextualizes the development of this shift in the scientific and cultural understanding of the body-bacterial relationship in the 20th and 21st centuries.

Rockefeller Institute for Medical Research (RIMR, later renamed Rockefeller University) microbiologist Rene Dubos played a key role in shifting the discussion of the microbe-human relationship from pure pathogenic terms to ecological terms. He holds a particularly interesting place in this history because of the various scientific areas that he straddled—soil microbiology, infectious disease research, and ecology. The research that he did on, as he called it, the "ecosystem," that is the digestive tract, brought all of these disparate research interests to bear on the problem of nonpathogenic microbes.

Dubos was of course, not alone in re-conceptualizing the human body in ecological terms. As early as the 1930s, dental researchers were thinking about the study of the mouth in ecological terms.[5] In 1965, microbiologist Mary Marples published a massive tome on *The Ecology of the Human Skin*, which had a profound impact on dermatological research.[6] Gynecologists were fully engaged with the "microbial ecology of the vagina" by the 1970s.[7] Dubos, for his part, declared in a paper published in the *American Journal of Medical Sciences* in 1964, that the digestive tract *was* an ecosystem, and had been investigating the gastrointestinal tract along these lines for several years beforehand.[8] Except unlike these other areas, Dubos was not a medical microbiologist working within the confines of a clinical specialty. He was a premiere researcher at one of the most storied research institutions in the world. Why then, did Dubos pursue this research agenda, and how did he do it?

The Rockefeller Archive Center's (RAC) Dubos Collection contains letters, manuscripts, speeches, grant applications, book reviews, and other documents that elucidate this transitional chapter in Dubos' career, in which he moved from renowned microbiologist famed for his fundamental work in the development of antibiotics to environmentalist icon and iconoclast. His work in what has come to be known as human microbial ecology helped shape the questions that are now being posed by the Human Microbiome Project and others. Dubos' research is an interesting node at which to examine how concepts about the medical body, experimental models, ecology and microbiology came to be linked together in the laboratory and theoretically. For him, his work addressed a fundamental question about 20th century biomedicine—what does the reductionist imperative in science leave out in our attempts to understand life? How can we address this shortcoming experimentally?

Researchers like Dr. Arthur Isaac Kendall, who was Christian Herter's research assistant for a time in the early years of the RIMR, had been investigating "intestinal bacteriology" and nonpathogenic bacterial-human relationships as early as the 1910s.[9] Like Kendall and some of the other pioneers of what had been called "intestinal bacteriology," Dubos did not come to the study of the bacteriology from medicine, but rather from agriculture. Dubos earned his Ph.D. in microbiology under Selman Waksman, the esteemed soil microbiologist, in the department of biochemistry and microbiology at Rutgers, and had studied before then in a French school of agriculture.[10] Waksman, who had come to soil microbiology through a background in farming, had been hugely influenced by the agricultural microbiologist Sergei Winogradsky. Both Winogradsky and Waksman were interested in the chemical processes of living systems.

Indeed, Dubos had found microbiology boring, with its focus on taxonomy, until he read an article by Winogradsky in 1924 that suggested a whole new approach to microbiology to the young Dubos. Waksman, who has been called the father of microbial ecology, chided microbiologists for focusing so heavily on the technique of pure culture, insisting instead on the importance of studying bacteria *in context*—shifting the focus to their relations in nature and their interactions with their environment.[11] This became a central principle in Dubos' work for the rest of his career; he claimed later that his intellectual life had begun with these ideas.[12] When Dubos turned to medical microbiology on the advice of Waksman and took a job at RIRM, he brought this orientation with him.

In the late 1950s, Dubos was chafing under the constraints of traditional scientific research. He had come to the conclusion that "the very success of the reductionist approach has led to the neglect of some of the most important and probably the most characteristic aspects of human life."[13] By the beginning of the 1960s he had begun to develop non-reductionist research

programs that targeted these "most characteristic aspects" of human life. He aimed at the mundane, in other words—and one of the key ways in which he did this was to turn the study of pathology on its head—to take a different tack from most of his peers studying medical microbiology.

In his book *Mirage of Health*, published in 1959, Dubos previewed where some of his thinking was with respect to microbes. In a review of the book, Dr. Leonard Berman, of the Georgetown University Medical Center, led with Dubos' "different viewpoint on germs." One of the key arguments of the book according to Berman was the notion that there was "a balance of forces in nature" that needed to be respected. Dubos' book stressed how the eradication of a type of bacterium living peacefully with humans could lead to "a power vacuum into which far more dangerous invaders would be pulled."[14]

Dubos had been thinking about this for some time, sparked in part by his wife's tuberculosis. In the mid-1950s, Dubos gave several talks and wrote several papers on the notion of "living at peace with infection," highlighting how most humans carry within them microbes that are capable of becoming virulent pathogens. The key issue was determining what the conditions were that turned infection into disease. He argued that a new way of conceptualizing the germ-body relationship was needed to understand this, drawing on concepts from Charles Darwin and Claude Bernard in describing evolutionary adaptation.[15]

Dubos took this argument into the laboratory in the late 1950s. He knew from his training in soil microbiology that the microbial world was much more diverse and richer than medical microbiology, with its laser focus on pathogens, imagined. The problem was that they were not readily cultivable with the pure culture techniques and media available because of their *in vivo* and highly specific habitats. The first order of business was to therefore, identify as many species

as possible by developing histological and selective culturing techniques in the lab to visualize and isolate them. In 1961, Dubos changed the name of his RIMR laboratory from Bacteriology and Pathology to Environmental Biomedicine, a nod to his holistic view of biomedical research and biomedical function. The following year, Dubos deemed his pilot studies of the indigenous microbial flora of mice worthy of additional funding sources, despite the misgivings of his colleagues.[16] He applied for, and won funding, for his studies on the relationship of the indigenous microbial flora and infection, with funding from the Health Research Council of the City of New York in 1962; the following year he received funding for a related project on the indigenous gut flora ("Intestinal Flora, Growth, and Resistance to Disease") from the National Research Council.[17] Both grants would support this research through the entire decade and into the 1970s.

The studies aimed to investigate several questions and hunches that Dubos had. First, he sought to "determine the effects of the indigenous microbial flora on the susceptibility of experimental animals to various types of infection" and whether the intestinal flora was "of special importance in the determining of the physiological characteristics" of host animals.[18] Through the 1960s and into the early 1970s, Dubos' lab developed practical means for "the quantitative enumeration on a large scale of the anaerobic bacterial flora in the digestive tract," as well as new culture media for specific intestinal bacteria that became "routinely used" in studies of intestinal and anaerobic bacteria. Dubos and his collaborators also experimented on the relationship between malnutrition and infection using germ free mice and experimental mice, with well-defined bacterial profiles that had been developed at the RIMR.

In late June 1966, at a meeting of the American Proctologic Society in Cleveland, Dubos delivered the Louis J. Hirschman Memorial Lecture on the indigenous flora of the

gastrointestinal tract. Dubos described his goal in studying the indigenous flora and claimed that medical microbiology had come up short in infectious diseases with latency periods, because it had focused only on potential pathogens when studying the normal flora. They had missed the fact that "the symbiotic species are of at least equal importance," because they were "essential to the well-being of the host."[19] Rather than just being nonentities as had often been assumed—harmless hangers on that could be ignored in the study of medicine—they were actually implicated in the physiology of their human or animal hosts. These microbes, Dubos claimed, "elicit[ed] histologic and physiologic responses, which constitute the normal healthy state of the gastrointestinal tract."[20]

In the lecture, Dubos hinted at the vast number of symbiotic relationships found in nature, referencing a few bibliographic items for the reader to explore further, and clearly placing the gastrointestinal tract alongside other *biological*—in other words, not just *medical*—phenomena. These symbiotic relationships were of two kinds, he claimed. There were the autocthonous microbes, that pointed to a well-established association with the host developed over a long period of *co-evolution*. These were pure non-pathogens. Then there were microbes that could *become* pathogenic as long as there was a biological equilibrium in the gut that kept them at bay. These, Dubos argued, were examples of microbes that had not yet had the time to *co-evolve* into pure non-pathogens. Dubos and his collaborators were most interested in the autochthonous flora, claiming that it had a role to play in the *development* of the host.

Towards the end of his talk, Dubos struck a lofty tone to emphasize the theoretical agenda he had become committed to: "Man becomes what he is through his responses to the forces of the environment in which he functions."[21] So here Dubos was making a striking argument about the function of the body to practicing proctologists, in evolutionary as well as

ecological terms. It was an implicit challenge to the fundamentals of how the body was understood and how its development was taught, studied, and conceptualized in modern medicine.

What Dubos saw in his laboratory led directly to ideas he expounded upon in *Man Adapting* in 1965 (based on a series of lectures given at Yale), and then later in its sequel, *So Human An Animal*, which won him a Pulitzer Prize in 1969. The changes wrought on the bodies of experimental mice, most drastically viewed in the cecum, suggested that the body flora had a morphological role as well as an immunological protective and nutritional role in the host. The key idea was the adaptation of the individual in its environment—the ecological present produced by host and flora as an "evolutionary equilibrium."

Looking back at his career in the 1980s, Dubos mused on his work and its relationship to ecology. "I now realize," he wrote, that "ever since I began my professional life as an experimental biologist in 1924, I have always looked at problems from an ecological point of view by placing most emphasis not on the living things themselves, but rather on their interrelationships and on their interplay with surroundings and events." [22] In fact, his early publications punctuated this point—he had published on the influence of environmental conditions on the microbial decomposition of cellulose in the soil in the, at the time, fairly new journal *Ecology* in 1928.[23] This ecological approach is poised to revolutionize biomedicine in the coming years, and was in part, developed by Dubos' prescient theoretical and experimental explorations of an *ecological body.* Dubos provided some of the foundational concepts and technical leg-work for researchers working with highly technical tools, National Institutes of Health (NIH) funding, and a high level of visibility today, as they try to answer the same questions he posed decades ago.

In closing, I would like to thank the wonderful staff of the Rockefeller Archive Center for all of their help, guidance and patience! A Grant-in-Aid provided an invaluable month of research support in a lovely setting that will be an important part of my dissertation.

ENDNOTES:

[1] "NIH Launches Human Microbiome Project," *NIH News*, Wednesday, December 19, 2007. http://www.nih.gov/news/pr/dec2007/od-19.htm. For press coverage, see for example, Gina Kolata, "In Good Health? Thank Your 100 Trillion Bacteria." *NY Times*, June 13, 2012; Carl Zimmer, "How Microbes Defend and Define Us." *NY Times*, July 12, 2010; Ron Winslow and Jonathan D. Rockoff, "Gene Map of Body's Microbes Is New Health Tool." *Wall Street Journal*, June 14, 2012. For scientific articles in major publications, see for example, R. Ley, et. al. "Microbial ecology: Human Gut Microbes Associated with Obesity." *Nature* 444, p. 1022-1023, December 21, 2006; Turnbaugh, Peter J, et. al. "The Human Microbiome Project." *Nature* 449: 7164 (October 18, 2007), pp. 804-810; and Gill, et. al. "Metagenomic Analysis of the Human Distal Gut Microbiome." *Science* New York, New York, 312: 5778 (June 2, 2007), pp. 1355-1359.

[2] The "Microbiome" is the collective genomes of these microorganisms in and on the human body.

[3] See Stefan Helmreich, *Alien Ocean: Anthropological Voyages in Microbial Seas*. Los Angeles: University of California Press, 2009.

[4] Turnbaugh, Peter J, et. al. "The Human Microbiome Project." *Nature* 449: 7164 (October 18, 2007), pp. 804-810.

[5] Appleton, J.L.T, "The Problems of Oral Microbiology as Problems in Ecology." In George M. Anderson, editor, *Proceedings of the Dental Centenary Celebration*. Chicago, Illinois: American Dental Association, 1940, pp. 282–293.

[6] Mary J. Marples, *The Ecology of the Human Skin*. Springfield, Illinois: Charles C. Thomas, 1965.

[7] W. J. Brown, "Microbial Ecology of the Normal Vagina." In E.S.E. Hafez and T.N. Evans, editors, *The Human Vagina*. Elsevier: North Holland, Amsterdam, 1978.

[8] Rene Dubos and Russell Schaedler, "The Digestive Tract as an Ecosystem." *American Journal of Medical Sciences* 248 (1964), p. 267.

[9] RU RG 110.2, Scientific Directors Minutes 1901-1911, Box 2.

[10] Rene Dubos, "The Infinitely Small: Microorganisms in Human Life and in Science," prepared for *Scientists Speak: Biology*. New York, New York: Harcourt Brace and Company, 1959, RU Rene Dubos Collection, 450 D851, Box 26, Folder 22.

[11] Sergei Winogradsky, "Sur la methode directe dans l'étude microbiologique du sol." *Chim. et Indus.* (Paris) 11 (1924), pp. 1-8.

[12] Rene Dubos, "Pasteur's Dilemma: The Road Not Taken" (American Society of Microbiology Lecture, Diamond Jubilee Symposium, Apr 2, 1974). RU Rene Dubos Collection, 450 D851 Box 28, Folder 13. Also see "Rene Jules Dubos," *Biographical Memoirs National Academy of Sciences* 58 (1989), 133-61, p. 135.
[13] Quoted in Carol Moberg, *Rene Dubos: Friend of the Good Earth, Microbiologist, Medical Scientist, Environmentalist* (ASM Press: 2005), 122.
[14] Leonard B. Berman, "Different Viewpoint on Germs," review of *Mirage of Health* by Rene Dubos, published in 1959, publication unknown. RU Rene Dubos Collection, 450 D851, Box 51, Folder 11.
[15] See, for example, Rene Dubos, "On Living at Peace with Infection," (Address before American Foundation, November 15, 1955), RU Rene Dubos Collection, 450 D851, Box 26, Folder 3; Rene Dubos, "The Germ Theory Revisited" (Lecture given at University of California, Berkeley in March 25, 1954), RU Rene Dubos Collection, 450 D851, Box 118, Folder 4, "Conferences and Lectures;" and "Infection into Disease" (manuscript from ca. 1956), RU Rene Dubos Collection, 450 D851, Box 26, Folder 6.
[16] Moberg, Rene Dubos, p. 123.
[17] RU Rene Dubos Collection, 450 D851, Box 54, Folder 1, Health Research Grant 1962-1975 and RU Rene Dubos Collection, 450 D851, Box 54, Folder 2, National Research Council, Health, Education and Welfare Grant 1963-1974.
[18] RU Rene Dubos Collection, 450 D851, Box 54, Folder 1, Health Research Grant 1962-1975 and RU Rene Dubos Collection, 450 D851, Box 54, Folder 2, National Research Council, Health, Education and Welfare Grant 1963-1974.
[19]Rene Dubos, Russell Schaedler, and Dwayne Savage, "The Indigenous Flora of the Gastrointestinal Tract." *Disease of the Colon and Rectum* 10 (1966), pp. 23-33. Delivered June 21, 1966, p. 23.
[20] Dubos, "The Indigenous Flora of the GI Tract," p. 23.
[21] Dubos, "The Indigenous Flora of the GI Tract," p. 29.
[22] RU Rene Dubos Collection 450 D851, Box 31, Folder 14, Autobiographical Manuscript.
[23] Rene Dubos, "Influence of Environmental Conditions on the Activities of Cellulose Decomposing Organisms in the Soil." *Ecology* 9 (1928), pp. 12-27.