A rendezvous with our microbes

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On November 2–3, 2009 an international group of scientists representing multiple disciplines gathered to consider the current state of our understanding of the symbiotic and beneficial relationships between microbes and humans and to define the challenges, gaps in knowledge, and opportunities that this exciting field of study now offers.

A number of adjectives come to mind when describing the subject of microbes and health, ranging from *ancient* and *historic*, to *integrative* and *interdisciplinary*, to *timely* and *pressing*. Coexistence and coevolution with microbes has been a theme of life on Earth for all metazoans past and present. Historically, the discovery that microbes are an integral part of us was made as soon as Antonie van Leeuwenhoek peered through his microscope and examined dental plaque sampled from himself and others (without institutional review board approval!). His sense of awe and his early appreciation of the diversity our microbial partners were evident in the words he chose for a letter written to the Royal Society of London in September 1683:

> "Though my teeth are kept usually very clean, nevertheless, when I view them in a magnifying glass, I find growing between them a little white matter as thick as wetted flower: in this substance though I could not perceive any motion, I judged there might probably be living creatures. I therefore took some of this flower and mixed it either with pure rain water wherein were no animals, or else with some of my spittle (having no air bubbles to cause a motion in it) and then to my great surprise perceived that the aforesaid matter maintained very many small living animals, which moved themselves very extravagantly. . . ..The spittle of an old man that had lived soberly, had no animals in it; but the substance upon and between his teeth had a great many living creatures, swimming nimbler than I had hitherto seen. . ." (1)

The question of how microbes influence our health was posed in the very early days of the field microbiology. In his 1901 Wilde Lecture to the Manchester Literary and Philosophical Society (2), Eli Metchnikoff outlined differences in microbial diversity that exist among human body habitats, indicated the benefits that these communities may provide, and invoked Bouchard's term "auto-intoxication" (3) when describing how products of gut microbial community metabolism may produce deleterious effects on the host. His advocacy for consuming cultured sour milk containing "Bulgarian *Bacillus*" (now named *Lactobacillus delbrueckii* subsp. *bulgaricus*) was an early expression of a desire to use living microbes (probiotics) to influence the properties of the intestinal microbiota in ways that enhanced well-being.

A century ago, Arthur Kendall published an article in the *Journal of Biological Chemistry* noting that:

> "The alimentary canal may be regarded from the point of view of bacterial processes within it, as a singularly perfect incubator.... The multiplicity of types and variety of physiological requirements of this intestinal flora are indications of the excellence of the incubator and a strong reminder of the influence which the unrestrained activity of these organisms might conceivably exercise upon the general condition of the host. While it must be admitted that the purely academic methods of research have resulted in scores of more or less complete morphological and cultural descriptions of bacteria of intestinal origin, this knowledge is fragmentary and unclassified. It is devoid of data which would permit one to correlate the presence of these organisms with the diet or condition of the host, or even to form a judgment concerning their numerical relations with other intestinal organisms. As this food passes through the alimentary canal. . .at different levels of the tract it is decomposed in part by various types of bacteria. The predominating types of bacteria which take part in the decomposition are determined largely by the nature of the diet....There is a parallelism between the nature of the diet and the character of the bacterial types represented in the intestinal and fecal flora. Hitherto this correlation between diet, intestinal flora and end products has been largely overlooked." (4; see also ref. 5)

His comments could easily be part of a conversation occurring today in laboratories that are using next-generation DNA sequencers, high-field NMR instruments, and/or a variety of mass spectrometers to understand how our gut microbiomes are being shaped by our rapidly changing and varied diets.

More than a half a century ago, methods were developed to rear and propagate a variety of mammalian species, notably rats and mice, under germ-free conditions. Comparing the properties of germ-free animals with those of their conventionally raised, microbe-laden counterparts, or assessing the effects of deliberately introducing microbial communities from conventionally raised donors into germ-free recipients at various points in postnatal life and adulthood provided a way to determine how communities from various body habitats shape host biology. At the same time that these gnotobiotic approaches were being developed by people like Bengt Gustafsson, James Reniers, P. C. Trexler, Julian Pleasants, Bernard Wostmann, Masasumi Miyakawa, Edward Balish, Tore Midvedt, and Morris Pollard (6), the field of anaerobic microbiology was blossoming, with heroic efforts to culture (and name) previously uncultured organisms. The challenge was inspiring but sobering: investigators kept noting that the great majority of microbes present in many environmental communities could not be cultured in the laboratory (7). All of these events sponsored conversations, such as those that occurred during meetings of the Armed Forces Epidemiological Board (http://history.amedd.army.mil/booksdocs/itsfirst50yrs/section1.4.html), where investigators like René Dubos, Russell Schaedler, Rolf Freter, and Dwayne Savage spoke of the importance of obtaining deeper knowledge about the normal (intestinal) microbiota and of its importance to medicine.

Deeper understanding was made possible in part with the advent of DNA sequencing and PCR, allowing culture-independent methods targeting the small subunit ribosomal RNA gene to more comprehensively define the phylogenetic structures of microbial communities. A profusion of new microbial lineages and even a new domain of life (the Archaea) were discovered in pioneering work by Carl Woese, Norman Pace, and their colleagues and students (8–10). With increases in DNA sequencing capacity,

This paper results from the Arthur M. Sackler Colloquium of the National Academy of Sciences, "Microbes and Health" held November 2–3, 2009, at the Arnold and Mabel Beckman Center of the National Academies of Sciences and Engineering in Irvine, CA. The complete program and audio files of most presentations are available on the NAS Web site at http://www.nasonline.org/SACKLER_Microbes_and_Health.

Author contributions: J.I.G. and T.R.K. wrote the paper.

The authors declare no conflict of interest.

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reductions in sequencing costs, and the coevolution of computational tools to process the ever-increasing amount of data, the field of metagenomics was "born" (11). Microbiologists adopted a more ecologic focus whereby the functions of microbes were not only considered in monoculture but also in the context of the communities where they live and the habitats that these communities occupy, setting the stage for the next renaissance.

This field is inherently *integrative* and *interdisciplinary*. The task of identifying members of our microbial communities and characterizing their phylogenetic relationships requires integration of concepts and technologies from many disciplines, including genomics, evolutionary biology, population genetics, computational biology/bioinformatics, and statistics. The results are allowing us to see ourselves as a compendium of myriad species representing all three domains of life (and their viruses!). We are finding that for a given body habitat, very distinct collections of microbial (bacterial) species exist among individuals, even monozygotic twins. Nonetheless, these distinct species assemblages share common functional features in their community genomes (microbiomes). This point illustrates the importance of understanding how many of the principles gleaned from studying macroecosystems apply to our microbial communities. In macroecology, the neutral theory of community assembly predicts that most species in a given community will have the same general niche (profession), or adopt the broadest niche possible, endowing the community with functional redundancy. Applied to human microbial ecosystems, the theory predicts a high level of variation in phylogenetic types (phylotypes) that occupy a given habitat in different hosts, although the broad functions specified by community microbiomes are expected to be similar. Macroecology will help frame and interpret studies of our microbial ecosystems, ranging from how diversity is measured, to the relationship between diversity and productivity, to exploring the extent that priority effects determine microbial community assembly and composition, to basic approaches for sampling (the importance of time series studies to ascertain variation; balancing depth vs. breadth of sampling; the need to carefully define habitat characteristics; developing a taxonomy pipeline that can be scaled as sample collection and data generation accelerate dramatically; principles and best practices for sample archiving and distribution).

The number of genes in our microbiomes exceeds the number in our "human genome" by at least three orders of magnitude and likely considerably more. This means that we need to look at ourselves from "above" and from this supra-organismal perspective see that we are a mélange of microbial and human cells and genes, with our microbial parts providing us with attributes not encoded or expressed by our strictly defined *Homo sapiens* components. The concept that there is an inexorable flow of genes from parent to child is now being supplemented with an appreciation that there is a more variable, and modifiable, intra- and intergenerational flow of microbial genes that reflects the history of our contacts with our surroundings, and our lifestyles (our "personal historical ecosystems").

If we are a synthesis of coevolved microbial–human relationships, then a great challenge is to understand the time scale and drivers of this coevolution. Over the course of 2,000 centuries, modern *H. sapiens* evolved from hunter-gatherers, learned to cook with fire and hence sterilize components of our food, domesticated crops and animals, created cities, and increased our capacity to travel over great distances. In the last 2 centuries, we have engineered dramatic changes in our technology and markedly changed our environmental exposures (e.g., to water that is more sanitized, to infant formulas that are consumed in lieu of breast milk, and to antibiotics that we now synthesize). Therefore, we need to place our supra-organismal perspective in the context of the changes that have occurred and that are occurring within our richly varied cultural traditions. How do our family structures and practices, such as the handling of infants and our food choices, influence the flow of microbes and microbial genes among individuals within a generation and between generations of a kinship? The notion that our microbial communities are a reflection of our cultural practices and even of our family history and dynamics suggests a need to incorporate cultural anthropologists into this area of investigation to help design as well as interpret studies of human microbial ecology, its variation, and its relationship to health and disease. This theme of coevolution of disciplines, played out against the backdrop of a field that seeks to understand our coevolution with microbes, will hopefully occur when partnerships with other disciplines are forged to achieve a more comprehensive view of the factors that influence our "normal" intra- and interpersonal phenotypic and molecular variations, and our disease predispositions.

The emergence of this field is "*timely*." There is a dimension to human evolution—a microbial evolution—that is likely occurring at a very rapid rate as our societies undergo dramatic shifts in socioeconomic status and cultural norms, redistribution of populations from rural to urban areas, changes in patterns of food consumption, and alterations in our exposures to xenobiotics, ranging from antibiotics that we intentionally take to various potentially toxic compounds that we unintentionally or deliberately ingest. The differentiation of our human microbiomes among different groups of people, and the transmission of these microbiomes within and across generations, means that our microbial communities provide "snapshots" of how we have lived and how we are changing the way we live.

With the number of published descriptions of the organisms and genes that comprise our microbial communities increasing dramatically, an aspirational goal of this Colloquium was to emphasize the importance of moving quickly to well-designed informative studies of how these microbial communities normally operate, how they shape host physiology, and how they may be altered by probiotic, prebiotic, antibiotic, or other interventions. Achieving this goal means collaborations between those who model metabolic networks and chemists who are able to use targeted and nontargeted mass spectrometric, NMR, and other analytic approaches to delineate the metabolic underpinnings of cooperative and competitive relationships between microbial taxa, to delve into the specifics of microbial–host cometabolism, and to characterize the degree to which differences in our microbiomes are correlated with differences in our metabolic phenotypes (metabotypes) (12). Engineers are needed to devise new instruments for precise sampling of microbial communities from relatively inaccessible surfaces of our bodies. Advances are required to observe the operations of microbial communities in situ over varying physical scales, including micrometer-level resolution studies of microbial–microbial and microbial–host interactions. Information about the biogeography of microbial communities is critical to immunologists who wish to understand how members of microbial communities communicate with components of the innate and adaptive immune system. In fact, immunologists occupy a central node in this envisioned network of interacting disciplines, because varied approaches need to be applied to understand how the products of microbial metabolism are sensed by immune cells and shape the representation and expressed functions of immune cell subsets.

A number of the presentations made during this Colloquium illustrated how we are gaining new information about normal intra- and interpersonal variations in our microbial ecology, and coincidently new information about the potential roles

played by our microbiomes in affecting disease predisposition and disease pathogenesis. The opportunities for microbiome-directed diagnostics and therapeutics are great. Therapeutic strategies include (*i*) altering the representation or metabolic activities of taxa believed to be associated with disease or disease risk, (*ii*) identification of host genes that are manipulated by the microbiota that in turn can become therapeutic targets, and (*iii*) the use of a microbial species (or a consortium of species) and their metabolic products (or synthetic derivatives) as therapeutic agents. The Colloquium emphasized how experimental and computational advances could and should lead to construction of new translational medicine pipelines. These pipelines include preclinical components composed of relevant in vitro and in vivo models that could be used to identify and initially validate targets for microbiome-directed therapeutics (as well as microbiome-based biomarkers of therapeutic efficacy). Statistical integration of DNA-, RNA-, protein-, and metabolite-based datasets obtained from these models should inform the design and interpretation of human studies. At the same time recursive modeling of human datasets will help shape the design and interpretation of studies performed in preclinical models.

The output of all of this effort may be next-generation probiotic species [or collections of organisms, including those that approach the diversity of an intact, unfractionated microbiota (13)] to seed body habitats, new strategies for the reliable delivery of these organisms and their products to targeted locales in these habitats, and importantly, new tools for precisely defining their effects on various host populations. New sets of tools should also allow us to measure the impact of microbial communities on the nutritional value of foods and food ingredients, as well as on drug metabolism, bioavailability, and safety.

There is a "*pressing*" need for this field to advance rapidly given the great challenges we humans face to develop safe and healthy foods for a population that will increase to 9 billion by 2050 and to understand the link between evolving Western lifestyles and the dramatic global increase in diseases associated with these lifestyles (e.g., obesity, type II diabetes, and various immunopathologic states).

In summary, this Arthur M. Sackler Colloquium emphasized that we must continue to honor our microbial symbionts, and understand their benefits through the lenses of many disciplines.

ACKNOWLEDGMENTS. We thank Tore Midtvedt and Stanley Falkow for their personal recollections about events that helped shape this field of research.

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