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Microbial Biota of the Human Intestine: A Tribute to Some Pioneering Scientists

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Abstract

Research on the indigenous intestinal microbiota of man was initiated well before the end of the 19th Century. The work continued at a slow but steady pace throughout the first half of the 20th Century. Findings from the effort had little impact on medicine and other aspects of human biology, however, until the 6th decade of the 20th Century. During that decade, research in the area was begun by eight groups of investigators, each of which was led by one or two senior scientists with great experimental talent, creativity and foresight. Their findings added new dimension to knowledge of the microbiota and initiated an explosion of interest in research in the field that has continued to the present day. The research of the groups during the 1960's is described in this review as a tribute to the senior scientists who had such critical impact on this important field of study.

"At a time when microbiologic research has gained us so many laurels by following the research methods of Koch into the regions of the etiology and pathology of infectious diseases, it would appear to be a pointless and doubtful exercise to examine and disentangle the apparently randomly appearing bacteria in normal feces and the intestinal tract, a situation that seems controlled by a thousand coincidences. If I have nevertheless devoted myself now for a year virtually exclusively to this special study, it was with the conviction that the accurate knowledge of these conditions is essential, for the understanding of not only the physiology of digestion, ... , but also the pathology and therapy of microbial intestinal diseases." [Theodore Escherich, 1895. (31), translated by K.S. Bettelheim].

Introduction

The quotation above, over a century old, revealed much about its author. Escherich recognized the difficulty inherent in experimental efforts aimed at gaining understanding of how bacterial species by the hundreds interact with each other. He recognized as well that those microorganisms influence physiological properties of their human host including its capacity to resist certain diseases. He also understood that some of those bacteria are able to cause disease in their host. Those points; that intestinal microorganisms interact with each other, that they influence properties of their host and that they cause disease; have been since Escherich's day basic hypotheses guiding the efforts of students of the alimentary "microflora" (61,78-79).

In the 105 years since Escherich wrote his prescient comment, many scientists have labored to "examine and disentangle the apparently randomly appearing bacteria in the normal feces and the intestinal tract". This review is in part a salute to those individuals and their tireless efforts. It is in most part, however, an effort to highlight the work of those who initiated an explosion of interest in study of those "apparently randomly appearing bacteria" during the decade of the 1960s. To achieve that goal, I will concentrate on the findings of those investigators of the 1960s (Table 1) and give short shrift to those coming before and since that period. Fortunate for my purpose, research on the "flora" since the 1960s is a work in progress, the results of which are periodically examined and evaluated in reviews and other written works (61,78-79,91,109,128-129). Likewise, fortunate it is that Theodore Rosebury summarized in a book entitled "Microorganisms Indigenous to Man" the published findings of investigators working during the period spanning the late 19th century to the early 1960s (106).

At the time his book was published, Rosebury was a Professor in the Department of Bacteriology of the School of Dentistry at Washington University in St. Louis, Missouri. Writing the book had been for him a long labor of love. According to the Preface (106), he had first considered undertaking the project sometime between 1935 and 1938, started writing it in 1944 and completed it in time for its publication in 1962. As far back as 1928, he had been collecting references cited in it. In writing it, he stated, "In this book, I have undertaken to collect and organize the large and scattered literature on what microbiologists usually call the "normal flora" of man." The relevant literature indeed was "large and scattered". In each of his chapters, he organized, discussed and critically evaluated findings published in references by the dozens. The Author Index contained names by the hundreds. The book was, as a consequence, an effective review of findings from research on the "normal microflora" from its earliest days in the late 19th Century (quotation above) to the beginning of the 1960s. Rosebury stated in the Preface, "Nobody else, to my knowledge, has ever attempted such a book before." I believe that he was correct in holding that view.

The book gave only a preview of the knowledge of the "microflora" now available in the 21 st Century (see below). As large as was the "scattered literature", it provided information only sufficient for Rosebury to describe the then known microbial species that could be cultured in laboratory media from the body surfaces and the feces of normal

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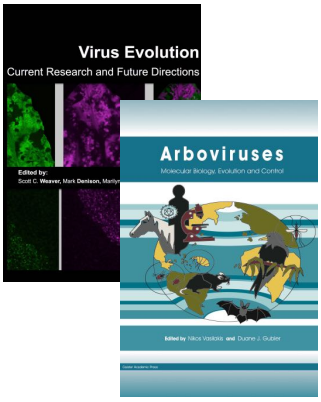

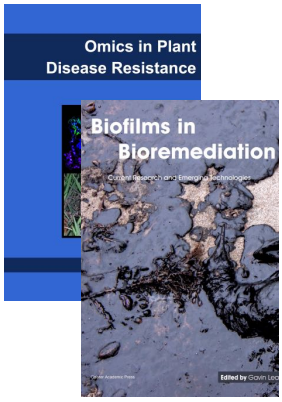

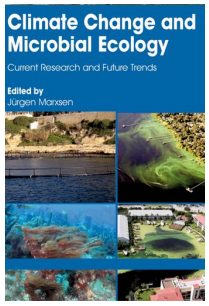

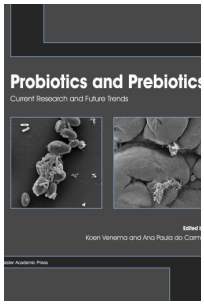

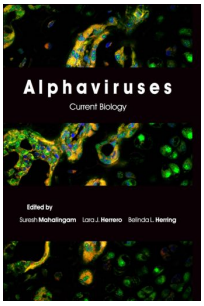

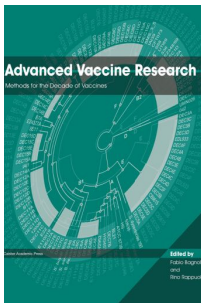

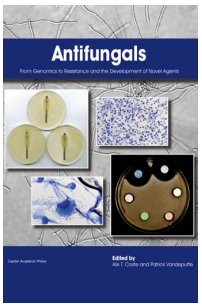

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Table 1. Investigators who contributed in the 1960s to the renewal of interest in research on the indigenous intestinal microbiota[a]

Country	Principal Investigators	Principal Investigators' Research Institute
FRANCE	P. Raibaud and. R. Ducleuzeau	Laboratoire d'Ecologie Microbienne, Institut National de Recherches Agronomiques, Centre National de Recherches Zootechniques, 78350 Jouy-en-Josas
GERMANY	H. Haenel	Institut fur Ernahrung, Bereich Mikrobiologie der Ernahrung, Potsdam-Rehbrucke
JAPAN	T. Mitsuoka	Institute of Physical and Chemical Research, Waka-shi, Saitama, and Department of Veterinary Microbiology, Faculty of Agriculture, University of Tokyo, Tokyo
SWEDEN	B. Gustaffsson and T. Midtvedt	Department of Germfree Research, Karolinska Institutet, S10401, Stockholm
UNITED KINGDOM	B.S. Draser	Department of Clinical Sciences, London School of Hygiene and Tropical Medicine, Keppel Street (Gower Street), London WC1E 7HT
UNITED KINGDOM	H.W.Smith	Animal Health Trust, Stock, Essex
UNITED STATES	R. Dubos and R.W. Schaedler	Rockefeller University, New York, New York, 10021
UNITED STATES	S. Gorbach	Department of Community Health, Division of Geographic Medicine and Infectious Diseases, Department of Medicine, Tufts New England Medical Center, Boston, Massachusetts 02111

[a]Alphabetized by country

humans (106). It fell short of providing the view of the "flora" and its ecology now available to us today. It was, nevertheless, a source of knowledge that helped during the 1960s to rejuvenate research on microorganisms found on the body surfaces of healthy humans. That research was undertaken at much the same time in the early 1960's by investigators in numerous laboratories over the world. Those investigators may not all have been aware of Rosebury's book. Indeed, some of them were already at work on projects involving the "flora" prior to the book' s publication (24,53,117). Therefore, all circumstances leading numerous scientists in laboratories in Europe, Japan and the United States to undertake concentrated study on related projects during a rather short period in the 6th decade of the 20th Century are unknown to me. Rosebury's book certainly contributed to the idea that such research was necessary and possible. It could not, however, have been the sole reason for the remarkable convergence of interest in the scientists who initiated and conducted the research during the 1960s.

Those scientists and their colleagues and students authored and published papers the findings in which added new dimension to the knowledge of the "normal microflora" of man. Interestingly, much of that new knowledge resulted from research on microorganisms colonizing the gastrointestinal tracts of mammals other than humans. Findings from that research provided a basis for study of the human intestinal "flora" in ways probably not imagined by Rosebury. They gave foundation as well to modern work on the ways in which microorganisms of the "flora" influence physiological properties of their mammalian hosts (79,87). The body of that research expanded our understanding of the "normal intestinal microflora", attracted many new investigators to the field and stimulated much research in the 1970s and 1980s, some of which continues to this day.

Given the state of knowledge at the time, the students of the intestinal "flora" during the 1960s were interested in the most basic issues concerning the "flora". They conducted research in which they cultured microorganisms from feces and intestinal content, identified the cultured organisms to genus and species, and estimated their populations. They also studied how microbial communities interact with the epithelium in the stomach and intestine, how those communities develop in neonates and how they are regulated in membership and population by dietary and environmental factors. This review will be confined to those issues only as they were studied in the 6th decade. As a consequence, I will neglect the large and important literature on methods perfected during the 1970s for culturing intestinal anaerobes from feces and intestinal samples and the use of modern methods for classifying those bacteria into taxonomic groups (1,33,34-36,63,97,108). Likewise, I will neglect the literature on how the "flora" impacts on its host' s anatomy, physiology and immunology. Much of that literature derives from studies begun as far back as the 1930s involving animals free of microorganisms except possibly certain viruses (germfree animals). It has often been reviewed (32). Also given short shrift are exciting new studies on how the intestinal "microflora" interacts at the biochemical level with intestinal and other body tissues ("cross-talk"). For a sense of the focus, direction and promise of these important new studies, a reader may wish to consult the publications of Bry et al (9), Franks et al (37), Millar et al (83), Suau et al (122), Tannock (127) and Wang et al (131).

Finally, I shall have little or nothing to say about intestinal bacteria of certain genera and oligosaccharides of certain molecular classes that are consumed as dietary supplements. Supplements containing microbial cells are called either "probiotics" (38-39,128) or "direct-fed microbials" (99) depending upon their use. Probiotics are consumed worldwide by humans (38-39,128); direct-fed microbials are fed to pets and animals grown for food (99). Supplements containing oligosaccharides are called "prebiotics" (7). These products are less widely available as commercial dietary substances than "probiotics" but are of much interest in research at this time (7,40,107).

Consumers of such products often believe that the microorganisms or the oligosaccharides in them have positive benefits on health. Those beliefs are supported by some experimental evidence but refuted by other findings. These issues and related ones have often been discussed and reviewed (39).

Much has been written in recent years about the "normal microflora" of the human small and large bowel. The authors of such works sometimes note historical underpinnings of the field (126). In doing so, they usually reach back to writings of pioneers working in the late 19th and early 20th centuries such as Metchnikoff (82) and Escherich (31). They reach that far back for a reason. The period of the late 19th and early 20th Centuries was a time of great ferment in microbiology and, as described by Rosebury (106), saw important developments in knowledge of the microbiology of the normal human body. Those developments were almost lost from the research and clinical arms of microbiology, however, for much of the 4th through the 6th decades of the 20th Century (106). During that period, numerous investigators conducted research involving the "flora" (106). Their findings had little impact on the field, however, until they were organized and evaluated by Rosebury and other students of the "flora" in the 1960s (104,106,).

Perhaps because he was a Professor in a School of Dentistry, Rosebury attributed the drought in interest in the "flora" through the first half of the 20 th Century to the rise of the germ theory of disease and the "exuberance" of microbiologists in pursuing the etiological agents of disease (106). It may also have been due to exuberance during the same period in use of pure culture technology for exploring bacterial metabolism and biochemistry, food spoilage and other important microbiological matters. One key reason in my personal view, however, was the use in most studies of microorganisms, and in particular bacteria, able to grow on laboratory media incubated in air. As is now well known (61,78-79), the vast majority of the microbial cells found in the normal human large bowel and in feces are of bacterial species able to multiply only on media incubated in atmospheres free of oxygen (anaerobes).

Anaerobic bacteria were discovered early in the history of modern microbiology (68). Unless they were either pathogens (e.g., *Clostridium perfringens*) or other anaerobic bacteria that could be exposed to and manipulated in air, however, they received little attention during the first half of the 20th Century. Most such bacteria simply could not be cultured in vitro by methods then in use. Effective methods were developed and put to use in the late 1940s and early 1950s for culturing anaerobes from the rumens of cattle (68). Those methods permitted culture of bacteria that are killed during exposure for brief periods (minutes; in some cases, seconds) to atmospheres containing oxygen (8). Availability of those methods, and perhaps even more importantly recognition that they were necessary, were major factors in the dramatic growth of research on the intestinal "microflora" during the 1960s (see below).

The focus on aerobic microorganisms in the early decades of the last century led many scientists and medical personnel well into the 1970s (1980s?) to regard the intestinal bacterium named after Escherich (31), *Escherichia coli*, to be the predominating inhabitant of the human large bowel (personal experience). *E. coli* is a facultative organism able to grow on common laboratory media in atmospheres either free of or containing oxygen. The view that its populations dominated in the large bowel prevailed even though findings published as early as 1933 had revealed that anaerobic bacteria of the genus *Bacteroides* outnumbered *E. coli* in human feces (30). Of course, the focus of microbiologists on studies with pure cultures of aerobic bacteria and on *E. coli* in particular led to the modern revolution in molecular studies (129). Therefore, that focus cannot be decried. Nevertheless, it contributed to a lack of interest in research on and the downplaying of knowledge about the intestinal "microflora" during the first half of the 20th Century. Fortunately, Theodore Rosebury preserved for us much, if not all, of that early knowledge in his book (106) and gave me a starting point for my review.

Terminology

At this stage of our understanding of the microbial world, the term "normal microflora" is an awkward descriptor of microbial communities residing on body surfaces of plants and animals. The term is commonly used in medical circles to distinguish microbial agents of disease from other microorganisms cultured from body surfaces. It is, however, a term without meaning in our modern understanding of the ecology of the intestinal "microflora" (109). In ecological terms, microorganisms forming stable communities native to a particular habitat are "indigenous" (autochthonous) to that habitat. Rosebury recognized that nomenclature when he entitled his book, "Microorganisms Indigenous to Man" and when he mentioned in the book "autochthonous" microorganisms when describing the "microflora" (106). He did not explain, however, why he considered those terms to be suitable replacements for "normal" in describing microorganisms recovered from the body surfaces and feces of normal humans. He may have been handicapped in explaining the issue by lack of evidence. At the time, findings supporting a hypothesis that organisms on the surfaces of the normal human body conform to laws of ecology as surely as do plants and animals were thin and incomplete. That case was easier to make in the mid-1970s (110). Pending that discussion, I will emphasize at this point that a composite descriptor more accurate than "normal" for microorganisms colonizing normal human body surfaces is either "autochthonous" or "indigenous" (26).

Use of the word "microflora" may be even more problematic than use of "normal". Microbiologists now recognize that prokaryotic microorganisms should be classified in taxonomic domains other than those in which are classified eukaryotic microorganisms, plants and animals (Eukarya). That classification is based upon analysis of certain DNA sequences and is now accepted by authors of most major textbooks in microbiology (112). It assigns prokaryotic microorganisms to not one, but two domains, Bacteria and Archaea (112). Biologists have long been comfortable in referring to communities of animals and plants in the kingdom Eukarya as "fauna" and "flora", respectively. Long before their classification into the new

domains, microorganisms of most taxonomic groups had been thought to be plants. Their natural communities were, therefore, described as "flora". That term is now at best awkward and at worst incorrect when used in reference to microbial communities. However, no specific alternative to it has emerged. As a consequence, some investigators use the word "biota".

"Biota" is a more encompassing term than either "flora" or "fauna" as a descriptor of communities of biological organisms. Rosebury used it, without explanation, in describing microbial communities on human body surfaces (106). Possibly taking their cue from Rosebury, Dubos and his colleagues suggested many years ago, that it was a good term for referring to indigenous microbial communities in the intestine (26). When it is used in place of "flora", the indigenous (autochthonous) "microflora" of man becomes the indigenous (autochthonous) microbiota of man. I shall use that terminology from this point forward.

Anyone with a rudimentary knowledge of Latin knows that the words "flora" and "biota" are plural. Using them as plural, however, has been awkward for me (112) writing about the intestinal microbiota. Use of them with plural verb forms leads me to write in a stilted style that is too formal for conveying concepts and information about microorganisms living in the gut. Therefore, with apology to students of Latin, I will use them as singular words throughout this document.

Human Autochthonous Gastrointestinal Microbiota

Knowledge in the Year 1962

Rosebury used most of the chapters in his book for describing in some detail the major general classes of microorganisms known at the time to be culturable from human body surfaces (106). Those surfaces were the skin, the teeth and the mucosae of the mouth, upper respiratory tract, urogenital tract and large intestine as represented by feces. He then used the descriptions as a basis for outlining the biotas indigenous to those surfaces. He contended, based upon findings available to him, that biotas characteristic of the various surfaces differed not so much in kind (genera and species) as in quantitative dimension. In other words, he believed that microorganisms of the same general taxonomic groups could be found on most surfaces. He concluded, therefore, that the most "conspicuous point of difference between the characteristic biotas of the different regions is quantitative". That is, a particular bacterial species could be cultured from all the body surfaces but maintained higher populations on certain surfaces than on others. That view led him to characterize the biota of each surface with the phrase, "Microorganisms commonly found on healthy human body surfaces" (106, Table 2, pages 318-321). It also led him to use the word, "contaminated" in describing microorganisms appearing on the surfaces and in the feces of neonates after birth. As shall be seen, he may have taken a differing view had he been able to examine modern knowledge of the indigenous microbiota of humans.

Given his view at the time, however, he compared, with emphasis on the quantitative rather than qualitative dimension, the biota of the lower intestine to those of the skin, upper respiratory tract, mouth and genitourinary tract. He made the comparisons by giving estimates of populations of microorganisms of various major taxonomic groups. The data for the "lower intestine" (as represented by feces) are given in Table 2. Rosebury regarded these data to be evidence that the fecal microbiota was qualitatively little different from, for example, the upper respiratory biota, but that the population of an individual species was characteristic of the site of the body. The data from which he drew that conclusion (Table 2) were incomplete as compared to our newer knowledge and led him astray in his understanding of the indigenous microbiota of body surfaces. Nevertheless, they allowed certain important conclusions that are relevant to our current understanding of the biota. These are:

- every adult individual tested has a microbial biota,
- the biotas are composed of bacteria,
- most of the bacteria in the biotas are of species of genera of strict anaerobes such as *Bacteroides* and of metabolic anaerobes such as *Lactobacillus*,
- maximum bacteroides populations in adults are many fold higher than the populations of *E. coli* and coliform bacteria, and
- the biotas of almost all infants fed at the breast contain species of the genus *Bifidobacterium* in high populations.

Other conclusions could have been reached from the data. However, these points established the foundation for our modern view of the biota.

Knowledge in the Year 2000

Modern knowledge of the human indigenous gastrointestinal microbiota is the subject of textbooks (128) and has been reviewed in journals and analyzed in books devoted to the subject (42,61,78-79,109,112,127,129). As I have noted, my purpose in this review is to highlight how certain microbiologists working in the 1960s contributed to that knowledge and especially how they stimulated research in the area in the decades following that period (Table 1). Therefore, I will summarize the body of modern knowledge in some key descriptive points and use those points as a basis for discussing the findings in the 6th decade of those important students of the gastrointestinal microbiota. I will refer in those discussions to recent papers confirming and expanding the findings of those students. In a few cases where the investigators in the 1960s had not reported evidence relevant to a modern point, reference is made to works of other authors.

- The indigenous gastrointestinal microbiota of normal humans is composed of communities of anaerobic bacteria.

- These microorganisms can be classified in hundreds of species in as many as 50 genera in the domain Bacteria. Some produce methane and are classified in the domain Archaea (84).
- The indigenous communities reside in the distal small and large intestine.
- Transient bacteria can be cultured from feces and from gastric and intestinal content. These organisms derive from ingesta and habitats above the gastrointestinal tract.
- The combined populations in the indigenous communities in the small and large bowel can be enormous, exceeding one hundred trillion bacterial cells (109).
- This enormous bacterial mass forms about one-half of the fecal mass (121).
- The communities establish in the neonate in a pattern that can be recognized to be an ecological succession.
- Some species resident in intestinal habitats form communities in the intestinal mucous gel lining the intestinal epithelium.
- All members of the gastrointestinal communities obey ecological laws governing biological organisms; all communities are regulated by numerous complex forces including some exerted by the host (e.g., gastric pH) and some they exert themselves (e.g., colonization resistance).
- The combined bacterial population, the microbiota, can be viewed as an organ of the body that is indispensable for life under ordinary conditions.

Table 2. Microorganisms Commonly Found in the Lower Intestine as Reported by Rosebury (106)

Major Group	Species or General Class	Population in Feces	
		Infant	Adult
Gram-positive Cocci	Coagulase-negative staphylococci	31-59[a]	(2-4/g)[b]
	Coagulase-positive staphylococci	10-93	++[c]
	Streptococcus mitis and undifferentiated γ streptococci	14-32	+
	Streptococcus salivarius	0-6	+
	Enterococci or Group D streptococci	87 (6-9/g)	100 (3-8/g)
	Streptococcus pyogenes	0.7-19	16
	Anaerobic streptococci	+	
Gram-positive Bacilli	Lactobacilli	60	(0-7/g)
	Aerobic corynebacteria	10-21	6
	Mycobacteria	+	
	Clostridium perfringens and other species	13-19	25-35
	Clostridium tetani	1-35	
	Actinomyces (now *Bifidobacterium*) *bifidus* (7-11/g)	15-16	90
Aerobic Gram-negative			
	Bacilli	86-100	100
	Undifferentiated “coliforms”	(7-9/g)	(5-8/g)
	Escherichia coli	67-99	100
	“Intermediates”	28-52	
	Klebsiella aerogenes	19-48	33-68
	Proteus mirabilis, other species	48	5-53 (0-6/g)
	Pseudomonas aeruginosa	+	3-11
	Alkaligenes faecalis	0-2.1	+
	Moraxella and *Mima* species	+	
Anaerobic Gram-negative			
	Bacilli, Vibrios. And Spirochetes		
	Bacteroides fragilis and other species	100	(7-10/g)
	Bacteroides nigrescens	+	
	Fusobacterium fusiforme	+	
	Fusobacterium girans	+	
	Treponema dentium and *Borrelia refringens*	18	28
Fungi	*Candida albicans*	+	14-31 (0-4/g)
	Other candidas	1-12	
Protozoa	Species of several genera	8.0-32.1	

[a]Percentage of individuals yielding bacterium in culture
[b]Log_{10} estimated number of bacteria per gram of feces
[c]+, commonly cultured; ++, prominent in culture

B.S. Drasar and Colleagues

" ... the faecal microflora is not primarily controlled by the presence of undigested food residues in the large bowel" (18).

B.S. Drasar came to research on the intestinal microbiota through a focus on noninfectious diseases of the human intestine (16). He was a microbiologist with interest in the etiology, unknown at the time, of diseases of man such as intestinal cancer, achlorhydria, malabsorption syndromes and inflammatory diseases of the bowel. He and his colleagues did their experimental work, therefore, with humans as subjects. He recognized early the need for methods effective in culturing anaerobic bacteria from human feces and intestinal content (14,16,19). He worked with his colleagues in developing methods for sampling fluids for microbial culturing from the human stomach and small bowel. They then used the methods for estimating populations of bacteria of various species in samples of gastric and intestinal fluids and feces from normal individuals and patients with various gastrointestinal disorders (16,19).

In one of their earliest papers (19), he and his co-workers used their techniques for culturing organisms from gastric content of healthy persons and patients with achlorhydria (insufficient gastric acid). Their findings revealed that the stomachs of healthy individuals contain few culturable bacteria (less than 1000 per milliliter of fluid) of species representative and presumably deriving from the mouth and upper respiratory tract. The bacteria most commonly isolated were species of the gram-positives streptococci, staphylococci, and lactobacilli. By contrast, the stomachs of individuals with achlorhydria yielded in large populations bacteria of species of numerous genera including gram-negatives as well as gram-positives. These findings provided evidence for a long-standing hypothesis that hydrochloric acid in normal concentrations is a major factor in controlling bacterial populations in the human stomach (19).

These investigators were among the first to reveal that fluids in the small bowels of individuals with malabsorption syndromes (e.g., steatorhoea and nontropical and tropical sprue) often contain bacteria of the many genera and species characteristic of human feces (16). The organisms were usually present in high populations exceeding millions per milliliter of fluid. Such findings revealed how peristalsis in the upper small bowel controls the microbiota of the small bowel of healthy individuals.

Drasar and his colleagues also developed early in their careers interest in how indigenous bacteria might be involved in the etiology of intestinal cancer (15). That interest led them into studies of how certain enzymes expressed by intestinal bacteria catalyze deconjugation and other side-chain modifications in bile acids (17). Those studies led to what proved to be a long-standing effort of the group to understand how bile acids modified by such enzymes may be involved in the etiology of intestinal cancers (2,17,62).

Drasar and his co-workers have been influential and enthusiastic students of the human gastrointestinal microbiota. They have continued their research into the decades beyond the 1960s. Their work has been focussed on how the microbiota is influenced by disease and other factors (15,17,19) and how it may be involved in the etiology of certain important and intractable diseases (2,17). They were among the first of the students of the biota to provide evidence that the human diet is not, with few exceptions (7,40), a major factor in regulating bacterial populations in the human intestine (18). Likewise, they were among the first to contribute evidence on how gastric acidity and peristalsis function in controlling those populations (16,19). Their findings on bacterial populations in the normal and diseased stomach and small bowel have often been confirmed (42,80). Their findings and theories on how bile acids function in the etiology of intestinal cancer have stimulated numerous other scientists to conduct studies of chemical activities of the biota and their possible involvement in cancer (42). They should be commended for their powerful impact on the directions and focus of research into such important human problems.

Dubos, Schaedler And Their Colleagues

" ... some of the components of the intestinal flora have become symbiotic with their hosts in the course of evolutionary development ... (26)".

R. Dubos, R.W. Schaedler and their colleagues conducted research on the gastrointestinal microbiota of mice throughout the 1960s and well into the 1970s. They were inspired to begin the effort late in the 1950s by their discoveries and findings of others on how mice of a certain strain physiologically respond to dietary, environmental and chemical stimuli (114). The animals involved were called New Colony Swiss (NCS) mice. They were derived from Standard Swiss (SS) mice in 1959 by the staff of the facility for housing and breeding experimental animals of the Rockefeller Institute of Medical Research (now known as Rockefeller University) in New York City (Table 1). The NCS animals were derived from and eventually replaced the SS mice by techniques designed to eliminate from the mouse colony some common mouse pathogens. They were housed in specialized caging maintained in a facility in which all rooms and equipment were frequently treated with germicides and for which all air entering the rooms was filtered of microorganisms. They were bred and handled only by individuals wearing clothing designed to inhibit microorganisms on human clothing and body surfaces from entering the environment of the housing facility. Great care was taken to prevent microbial pathogens of mice and other animal species from entering the facility. Likewise, the animals were frequently tested to assure that they remained free of most mouse pathogens. They were known, therefore, as Specific Pathogen-Free or SPF mice (100).

SPF laboratory mice, now called "barrier-sustained" animals, are at the present time derived from germfree parents. They are available for experimental use from commercial suppliers and certain university laboratories. The NCS mice were derived, however, from parental animals with a biota on their body surfaces and carrying certain pathogens. The methods used in their development involved transfer into the aseptic environment of newborn

mice derived by Caesarian section from SS mothers. The newborn mice were fed milk by hand until they could eat solid diet. Such procedures are extreme in their difficulty for the operators; the rate of success is low. The scientists, J.B. Nelson and G.R. Collins (100), who used those methods to develop NCS mice probably created the first colony in the world of mice that had a gastrointestinal microbiota, were free of certain mouse pathogens and could be bred in quantity for major experimental work. Their findings inspired commercial firms and other universities to develop SPF colonies of mice and other animals. They deserve accolades for the effort.

Dubos, Schaedler and their colleagues began research with NCS mice almost as soon as they could obtain the animals in numbers sufficient for experiments (24,114). Findings from their work and from that of other individuals led them to conclude early in the 1960s that the NCS mice differed in numerous important characteristics from control SS mice housed and fed under identical conditions. NCS mothers bore on average more infants per litter than SS females. NCS animals grew faster and were larger by several grams at four and six weeks of age than SS mice. As contrasted to SS controls, NCS adults grew faster and better on diets containing wheat gluten low in lysine and threonine (24). They also survived bacterial endotoxin injected in doses that killed most SS adults within one or two days (114).

The fecal microbiota of NCS adults also differed in important ways from that of SS adults (25). It lacked E. coli and certain other coliform bacteria and other gram-negative facultative bacteria that could be cultured from feces of SS animals. It included, however, some coliform organisms that lacked in the SS feces. It also included certain lactic acid bacteria that could not be cultured from the latter mice.

Such data suggested to some observers at the time that NCS mice had experienced mutational changes in their genome during their derivation from SS mice (24). Study of such mutations, those individuals suggested, could yield findings explaining how NCS mice differed from SS animals in various physiological properties. Those suggestions were obviated, however, by the discovery that NCS mice housed with SS animals reverted to the conditions of the SS mice in every tested property including response to endotoxin and the species of bacteria that could be cultured from their stools (24,114). Such findings meant to Dubos and Schaedler that the gastrointestinal microbiota could profoundly influence the physiology of its animal host and set them on a course of study of the murine microbiota that lasted almost two decades. During that period, several students of the biota were trained in the laboratory. Some of these individuals went on to conduct their own research programs and train students in the area during the years following the 1960s (69-74,81,109-113,119-120).

Dubos, Schaedler and their fellows began focused research on the intestinal biota by developing media and methods for culturing bacteria from feces and intestinal samples (115). They used sampling methods that allowed for homogenizing both fecal and intestinal samples in diluent that limited their exposure to air. Their media were specifically composed to support growth of organisms from samples taken from laboratory rodents and were specialized for recovering bacteria able to grow either in air, in atmospheres with low oxygen concentrations or in atmospheres free of oxygen (115). Each such medium was incubated in a way that reflected its particular use. The methods were designed for high volume experimental work; each vessel used for incubating media for culturing anaerobic bacteria could accommodate petri dishes by the hundreds. The techniques were supplanted in the 1970s in the laboratory by more efficient and effective methods of culturing anaerobes (71,72). For the early 1960s, however, they were state of the art and most useful for estimating populations of intestinal bacteria of various taxonomic groups from samples from mice (115).

Dubos and Schaedler used their media and methods for culturing and estimating populations of bacteria of major taxonomic groups from feces and intestinal samples from SPF mice. Their interest in testing the hypothesis that the biota impacts on physiological properties of their animal host led them to focus early on how the biota develops in neonatal mice. Therefore, they cultured bacteria from samples taken not only from adult animals (26) but also from neonatal mice at various times after birth (115). Their findings from that work allowed them to make several important conclusions. They concluded that each major area (stomach, small intestine, cecum and large intestine) of the mouse gastrointestinal tract hosts its own microbial population composed of bacteria of taxonomic groups characteristic of the area. They also concluded that each of those various bacterial populations developed at a different time after birth forming a pattern now recognized to be an ecological succession (109,113,115). These important observations have often been confirmed for humans and other animal species (61,109,124)

Dubos, Schaedler and their colleagues made other important observations in their studies of how the unique bacterial populations of the stomach and small intestine could colonize those areas. Evidence available at the time, at least for humans, indicated that bacteria did not colonize the stomach and upper small intestine (see Drasar and Gorbach). Ingesta in those areas were moved at an overall rate exceeding that at which bacteria could multiply. Dubos, Schaedler and their colleagues conducted experiments, therefore, to learn how bacteria could establish and maintain populations in the stomach and small intestine of mice. Their findings confirmed, consolidated and extended earlier observations of other investigators that the bacteria associated with the epithelium, either by firmly attaching to a keratinized surface or by colonizing the mucous gel overlying the epithelium (26,113). Those findings inspired much research over the world in the decades following the 1960s. Findings from those many efforts led to the important conclusion that bacteria and other microorganisms adhere to epithelial surfaces or colonize mucous gels overlying such surfaces in establishing communities in the gastrointestinal tract (11,124). They also led to the conclusion that the capacity to associate with epithelial surfaces is a major factor in the ecology of indigenous gastrointestinal microbiotas in animals of all taxonomic groups including humans (111). The findings also augmented observations developing from the research of numerous investigators demonstrating that bacterial pathogens of the intestine associate with epithelial surfaces in causing disease (111).

Dubos, Schaedler and colleagues devoted much of their research in the later years of the 1960s and in the early 1970s to characterizing indigenous anaerobic bacteria colonizing the murine cecum and colon (50,71-72). Those efforts were similar in kind to research of other investigators on the anaerobes culturable from feces and other gastrointestinal samples of animals of many taxons including humans (33-34,97). Their work earlier in the decade was singular, however, in its impact on the field of study of the indigenous gastrointestinal microbiota.

Their major findings during the 1960s included the discoveries that the indigenous microbiota develops in neonatal mice in an ecological succession (109,115), and that indigenous microorganisms associate with epithelial surfaces in forming communities in the stomach and small and large intestine in mice (111,115). Those findings were important but were essentially expansion and confirmation of findings of others [see for example Porter and Rettger (102), and H.W. Smith, below]. Nevertheless, they stimulated other investigators to study during the 1970s and 1980s the succession of the biota in neonatal animals other than laboratory mice (124) and to explore in those animal models the capacity of indigenous microorganisms to associate with gastric and intestinal epithelial surfaces (103,111).

Dubos and Schaedler's most influential observations 1960s came in their recognizing that the gastrointestinal microbiota is symbiotic with its animal host, that it influences important physiological properties of that host, and that it consists of microbial species that obey all ecological laws governing the behavior and functions of biological organisms (26). Those observations and the experimental methods used to derive them should endure in history.

S.L. Gorbach and Colleagues

"The flora, ... , may be altered by preoperative starvation, oral antibiotics, cathartics, the effects of the underlying illness, and anesthesia" (116).

S.L. Gorbach and his co-workers were led in the 1960s into study of the gastrointestinal microbiota by their interest in human intestinal disease (42). They were among the influential students of the biota in the 1960s that confined their experimental work to humans as subjects (see also Drasar and Haenel). As a physician, Gorbach had interest in intestinal diseases ranging from those such as cholera (44) that are microbial in etiology to those such as nonspecific diarrheas of unknown etiology (45). He combined studies of the etiology of such diseases with study of how the intestinal biota might function in the etiology (45). He studied as well how the active diseases might influence the biota. Over many years following the 6th decade, he also examined how, bile acids, surgery, antibiotic therapy and other factors influence microbial populations in the intestine (42-44,46,49).

In conducting their research, Gorbach and his colleagues cultured bacteria from samples taken from the small intestine (48) as well as feces of their subjects (47). They were innovative in their efforts to obtain samples for culturing of fluids from various areas of the stomach and small and large intestine (48). They used for sampling of the fluids long flexible plastic tubes with bags containing mercury on their ends that were inserted into the tract through the mouth or nose. Use of such tubes for the purpose of sampling intestinal bacteria was novel for the time. Their methods for culturing organisms from the samples were, however, common for the time. They used both selective and nonselective growth media and jars containing gas free of oxygen for incubating media designed for culturing anaerobic bacteria. The methods made it difficult for them to prevent exposing the fluids and culture dishes to air and, therefore, limited their effectiveness in culturing anaerobes. Nevertheless, the results in the efforts were pioneering and contributed much to our knowledge about microorganisms in the human gastrointestinal canal.

Their studies yielded data indicating that the microbiota of stomach fluids is predominantly gram-positive and able to be cultured in air (see also Drasar). Less than 1000 organisms could be cultured from a milliliter of the gastric contents. Most of the organisms were streptococci, staphylococci, and lactobacilli presumably deriving from habitats above the stomach (48, and see also Drasar). Fluids from the proximal small intestine contained from 1000 to 10,000 bacterial cells. These organisms were also able to grow in air and were mostly gram-positives of the taxonomic groups found in the stomach (48). Occasionally, however, gram-negative bacteria such as coliforms and anaerobic bacteria could be cultured. Fluids from the distal small bowel yielded high populations of bacteria such as coliforms and the strict anaerobes *Bacteroides*, *Bifidobacterium*, *Fusobacterium* and *Clostridium* (42,47-48). These findings were compared with estimates of the populations of bacteria of various groups cultured from feces Such comparisons indicated that the bacteria found in the fluids of the distal small bowel were of taxonomic classes found in feces. Likewise, bacterial populations in the contents of the colon distal to the ileo-cecal junction were much the same as can be cultured from feces (47,48).

S. Gorbach, working with numerous other investigators continues to this day his interest in the intestinal microbiota. He has had particular interest in recent years in the possible function of the biota in the etiology of intestinal cancer (42). He has become a proponent of using probiotics containing lactobacilli to alter activity of certain enzymes expressed by members of the biota (42). The enzymes at issue may be involved in the etiology of certain tumors (41). Gorbach and his many colleagues have over the years since the 1960s continued to be effective and important students of the intestinal biota. They have been quick to incorporate new methods and techniques in their work. They have seen their important findings repeatedly confirmed by other investigators (80, and see Drasar). They have, as a consequence, continued since the 6th decade to publish useful and interesting knowledge about the human microbiota.

B. Gustafsson, T. Midvedt and their Colleagues

"The intestinal microflora in man constitutes by far the most cell-rich biochemically active 'organ' in the body." (88).

B.E. Gustafsson was interested in the 1950s and 1960s in germfree animals and how those animals differed physiologically and anatomically from animals colonized by indigenous microorganisms (51). He founded at the Karolinska Institute during the 1950s a facility for housing and caring for germfree animals and conducted research on certain of their physiological properties. He published numerous papers during that period containing data from studies in which he compared physiological and anatomical properties of germfree laboratory rodents and ex-germfree rodents associated with gastrointestinal microorganisms. Some of his most important papers contained data from experiments involving such animals in which he examined how microorganisms alter intestinal mucus (11) and components of bile such as bilirubin (51) and bile acids (52). He also built a large research group that has endured into the 21st Century.

That group under Gustafsson's leadership and later, after his too early death, under the inspired leadership of T. Midtvedt, expanded and refined the research with germfree animals. It also added studies of certain bacterial components of the indigenous microbiota. It kept its focus, however, on bacteria able to live in (64) and to digest (11) intestinal mucus and to transform bile acids and other substances found in intestinal content (64,88). Many papers resulted from the research in the 7th and 8th decades and even in the 1990s. A huge body of information was produced concerning how the gastointestinal microbiota influences molecular substances found in the alimentary tract (85-88). That body of information and experimental evidence from other investigators led Midtvedt and his colleagues to a major and important position concerning how animals interact with their gastrointestinal microbiota.

That position embodied the concept that conventional animals, including humans, are physiologically a composite of biochemical activities of animals cells making up their corpus and microbial cells making up their indigenous microbiotas (87-88). Properties exerted by the animal's cells and organs were called GACS or Germfree-Associated Characteristics. Such properties were found in animals whether or not they were colonized by a microbiota. Properties associated with the microbiota were called MACS or Microbe-Associated Characteristics. These were found only in animals with a microbiota (10,87-88). Those concepts led to the remarkable view expressed in the quotation given above.

The research initiated by B.E. Gustafsson and his colleagues and students and continued by T. Midtvedt and his students and fellows has contributed much to our understanding of the symbioses between animals and their gastrointestinal microbiotas. Findings from the work have stimulated investigators around the world to undertake studies of their own. Some of that experimental work has most recently involved use of the techniques and approaches of molecular biology (9,32). Use of that technology ensures that the findings of Gustafsson, Midtvedt and their associates will continue long into the future to impact our understanding of how the gastrointestinal microbiota functions as an organ of the human body.

H. Haenel and Colleagues

"Es bestand keine Korrelation der Mikrookologi zum Alter bsw. Geschlecht der Schulkinder." (3).

H. Haenel and his research fellows and colleagues began study of the human alimentary microbiota in the late 1950s and continued it through the 1960s. They came to the study through Haenel's interest in diarrheal disease in infants and school children (52). Their focus was on culturing microorganisms of certain taxonomic groups from feces of individuals in that age range. The number of subjects in each of their experimental studies was often large. For example, in one study in which they estimated populations of bacteria in the feces of school children, the estimates were made over two years for 265 children ranging in age from 6 to 16 years (3). Feces of some adults were also cultured for comparison. Over 502 stools were cultured. Such numbers should impress anyone who has ever cultured human fecal samples for estimating populations of bacteria. The research group conducted numerous such studies during the 1960s (54-60). Their findings contributed to our understanding of bacterial populations of bacteria in feces of children but were limited in scope by the methods used.

The group used established methods for culturing bacteria from and estimating their populations in feces. Those methods involved use of differential media containing dyes and antibacterial drugs. They also involved exposing the fecal samples and media to air and incubating media for culturing anaerobes in conventional chambers containing mixtures of gases other than oxygen. These methods limited the population estimates to organisms in certain broad major classification groups. As described for one study (3), the investigators estimated populations of so-called "total aerobes" meaning all bacteria able to grow when incubated in air. They then identified subsets of those organisms using methods acceptable for the time. For example, they reported estimates of populations of streptococci, coliforms and lactobacilli. They also estimated populations of "total anaerobes" (all bacteria culturable on a rich medium incubated anaerobically), gram-positive anaerobes of the so-called bifidus group (*Bifidobacterium bifidus*), Gram-negative anaerobes of the bacteroides group, and spore-formers identified as clostridia.

Other investigators have confirmed certain findings from the research of Haenel and his Fellows. Their estimates of "total aerobes" and the subsets of such bacteria (streptococci and coliforms) have often been repeated (61). Such has not been the case, however, for their estimates of the populations of the anaerobic bacteria. Their estimates of populations of the "bacteroides-group" for example rarely exceeded 1×10^9 organisms per gram of feces. When approaches used to culture such anaerobes include efforts to minimize or prevent exposure of the fecal specimens to air, the populations of such bacteria usually exceed 1×10^{10} organisms per gram of sample (34,97). Moreover, modern methods of culturing anaerobic bacteria

have demonstrated that the "bacteroides-group" includes bacteria not only of the genus *Bacteroides* but also of several other genera (97). Therefore, Haenel and his fellows' descriptions of the anaerobic members of the fecal biota of both children and adults are of limited value at this time.

Still, however, their body of research over the decade of the 1960s constitutes a valuable contribution to our knowledge of the intestinal microbiota. Few investigators have ever so painstakingly sampled so many fecal specimens. Few have ever employed such intensive statistical analysis of their data and comparative approaches to test the strength of their findings. Therefore, their approaches have been excellent guides for investigators analyzing the biota with stronger culture methods in the decades since the 1960s. Perhaps most importantly, their conclusions as exemplified by the quotation above that neither age, sex nor family history controls the populations of bacteria in the feces of school children and young adults have stood the test of time.

T. Mitsuoka and Colleagues

"There is a growing consensus that the composition of the intestinal flora is closely related to health" (91).

T. Mitsuoka is one of the most influential students of the gastrointestinal microbiota in the world. He and his students and fellows began to publish papers in the area in 1960 and continue to publish important works to this day. Their list of publications to date is astonishing in its length and range of subject matter (4-6,90-96,123,125). Their body of work spanning 40 years spans most important subjects in the field. Few investigators in the world studying any subject in laboratory science can claim a lifetime of success in scientific research as can Professor Mitsuoka. The Japanese people have recognized that success. He was awarded a number of years ago Japan's highest, most prestigious prize in science (Personal Communication). Accompanying the award was a citation for his outstanding achievements in study of the intestinal microbial biota. He may be the only investigator in that field in the world who has been awarded a significant scientific prize for his body of research.

Mitsuoka was an innovator in the field from the outset of his career. He had had the educational experiences required to earn him both D.V.M and Ph.D. degrees and was obviously informed about intestinal diseases. That interest and knowledge of the characteristics of germfree animals may have sparked his interest in the intestinal microbiota. He recognized from the start that the biota is an anaerobic one and began his work by innovating in the 1960s methods for culturing from and estimating the populations in feces of anaerobic bacteria as well as microorganisms able to grow in air (95). His methods involved selective and differential media, some of his own design, for estimating bacterial populations in feces of humans (4-6) and feces and intestinal content of animals other than humans (89-90,92,96,130). Efforts were made to limit exposure to air of fecal and intestinal samples. Methods for culturing anaerobes involved a unique and simple method, the "plate in bottle method" (95). The methods permitted Mitsuoka and his colleagues to culture bacteria of many differing taxonomic groups found in the samples. They also permitted them to isolate in pure culture many of the bacterial species cultured and to identify them to genus and species using sophisticated methods (4-6). These organisms are now maintained in a major culture collection (Japan Culture Collection) and are available for use to investigators over the world.

Mitsuoka and his colleagues used the methods during the 1960s to estimate the populations of bacteria of various taxonomic groups cultured from human and animal feces and intestinal content of birds and mammals of certain taxonomic groups (92). They were quick to focus, however, on bacteria of the genera *Lactobacillus* and *Bifidobacterium* and conducted studies in which they compared estimates of the populations of such bacteria in human and animal feces (89,90,93). They also conducted detailed studies of the fecal biota of humans with intense focus on the bifidobacterial populations in fecal samples taken in those studies (94). Mitsuoka was interested not only in how the biota was involved in overt disease, such as cancer, in animals and man, but also in conditions regarded as normal, such as aging (4-6,91). Those interests led him early into comparative qualitative and quantitative study of the fecal biota and in particular the bifidobacterial biota of humans of various age groups (89,93). In recent years, he has been a strong proponent of the concept that populations of lactobacilli and especially bifidobacteria are important factors in maintaining healthy balance in the microbiota (89,91). Findings from his recent research and from other investigators have begun to support his views about bifidobacteria and human health (126).

Tomotari Mitsuoka continues to be a major force in study of the human intestinal microbiota (91). He has retired from his position at Tokyo University (Table 1) but continues to publish important papers in the field and to influence investigators over the world to continue research in the area. He is in many ways a phenomenon with seemingly limitless energy and capacity to communicate his message that the microbiota is important to human health. The field of study of the microbiota owes him a debt that is too high to pay.

P. Raibaud, R. Ducluzeau and Colleagues

"La maitrise de ces facteurs constituerait un grand pas en avant dans le controle de las microflore aux premiers ages de la vie, periode au cors de laquelle cette microflore semble jouer un role primordial." (23)

This group began research into the intestinal microbiota early in the 1960s under the leadership of the great microbiologist P. Raibaud. It was later joined by R. Ducluzeau who brought to it interest and expertise in study of factors influencing how members of the gastrointestinal microbiota interact with each other. The group remains to this day one of the most prolific in publication and innovative in approach in research on the gastrointestinal microbiota (12-13,20-23,28-29,65-67,75-77). Its members were inspired in part by the findings of earlier students of the intestinal biota of humans and other animals. They were also inspired by findings of other investigators at their

institute who were studying certain physiological properties of germfree animals in comparison with comparable animals with a biota (104).

One of the earliest publications of the team dealt in detail with innovative approaches and methods for culturing bacteria including anaerobes from intestinal content of rats (104). That publication revealed the investigators' understanding of the need for restricting exposure of gastric and intestinal samples to air and for strict anaerobiosis in culturing bacteria from such samples. It also revealed their creativity in design of diluents for minimizing exposure of the samples to oxygen and oxidation-reduction potentials above a certain level and media for culturing anaerobic bacteria as well as species able to grow in air. It contained as well a thorough review of relevant literature in the field up to the early 1960s.

That important publication was accompanied by one in which Raibaud and colleagues demonstrated the utility of their methods and media (105). They reported in the latter paper data from experiments in which they had estimated populations of anaerobes of several major genera and of bacteria able to grow in air in samples of gastric and intestinal content from adult and infant rats. They compared the estimates for the animals of various ages and recognized in infants a developmental pattern now known to be an ecological succession (61,109, and see also Dubos and Schaedler and Smith). Those early findings gave their subsequent research a guiding spirit as exemplified by the quotation above. The group has always kept major focus on the biota during the earliest stages of ex-utero life.

The group continues its research to this day. They have studied almost every important issue concerning the gastrointestinal microbiota in experimental animals and humans, from how it is regulated in microbial composition and biochemical and genetic function, to its impact on certain physiological processes of the host. Their findings have confirmed their suggestion that the biota influences the physiology of humans and other mammals not only in adulthood, but also as such animals develop from birth (23). That concept underscores the view that the biota effectively functions as an organ of the body (88, and see Gustafsson and Midtvedt).

P. Raibaud and R. Ducluzeau have experienced great success in leading their scientific study of the biota in part at least because they have always surrounded themselves with creative and capable colleagues. In addition, they have contributed to important collaborative studies that have extended their research with neonates from experimental animals to humans (27,98,101). They deserve to be recognized as the leaders of one of the premier research groups in this field that began in the 6th decade of the 20th Century.

H. Williams Smith

" ... although the bacterial population of the faeces ... resembled that of the large intestine, great changes could occur in the population of the stomach and small intestine without any corresponding alteration being noted in the faeces." (118)

H. Williams Smith was a veterinarian interested in microbiological research. He initiated studies of the microbiota in the early 1960s. His focus was on how the biota develops in infant mammals. He was led into the studies by his interest in research on neonatal diarrhea in farm animals (117). He at first cultured bacteria from the feces of adult and neonatal animals at various times after birth (117). He later, however, included efforts to culture from neonates, also at various times after birth, microorganisms from all regions of the gastrointestinal canal (118). In the early studies he examined the feces of calves, lambs, piglets, and rabbits (117). He even examined in that effort the fecal biota of a human baby (117). In the later work, he examined the development of the biota in all regions of the gastrointestinal tract of animals of those same species, except the human. He also expanded the work to dogs, cats, guinea pigs, rats and domestic fowl (118).

He was not an innovator of methods for culturing bacteria from the feces and gastrointestinal tract. Indeed, he used techniques developed in the 1930s for estimating bacterial populations in natural samples, growth media developed in the 1950s for selective culture of bacterial pathogens from feces and methods for incubating anaerobes developed early in the 20th Century (117). The latter methods involved exposure to oxygen of feces and tissue samples and, as a consequence, were limited in usefulness for culturing intestinal anaerobes. Nevertheless, he was able to culture anaerobic bacteria of certain major broad classifications identified as "lactobacilli", "streptococci" and "bacteroides" and the specific anaerobic bacterium *Clostridium welchii* (*perfringens*) (117-118).

By estimating the populations of such bacteria in feces at various times after birth of the animals, he was able to learn in a limited way how the biota altered as the animals aged and changed physiologically (117-118). He learned that *E. coli*, *C. perfringens* and streptococci could be cultured soon after birth from the feces and samples of content taken from all areas of the gastrointestinal tract of the animals of most species. Those organisms were soon followed by lactobacilli, which in species other than dogs, cats and rabbits became the most common inhabitants of the content in the stomach and small intestine. These members of the biota were followed by bacteroides that were restricted to the large intestine but were the principal inhabitant in that area in most of the animals examined. Once the animals were weaned, the biota became distinctive in its microbial composition and characteristic of the animal species and changed little over time. However, anaerobes such bacteroides continued to predominate over the other bacterial classes in adults of all animals examined.

H. Williams Smith concluded from his findings that the bacterial biota of the feces was similar early in life in animals of all species he tested but progressively changed in composition until the animals were weaned (117-118). He

speculated that the biotas were similar among the species in early life because all mammals when young had the same diet (milk) and an alimentary tract similar in structure and function. He suggested that the developing biota became dissimilar as the animals age because their diets become increasingly dissimilar and their tracts diverge and become specialized in structure and function (118). He made attempts to test how diet influenced what bacterial species could be isolated from the biotas of adults of the individual mammalian species. His findings suggested that diet influences little the microbial composition of the biota in weaned animals (118).

Harry Williams-Smith was a careful scientist with excellent understanding of experimental method and control and much insight into how to approach study of the gastrointestinal microbiota. He made important observations in the field that have stood the test of time in spite of the fact that his methods were not the most powerful for yielding full understanding of the microorganisms resident in the intestinal canal. In painstaking work involving animal dissection and culture of microorganisms, he showed the way to other investigators by demonstrating that feces are limited in what they can reveal about the biota in the stomach and small intestine. His studies of the biota in various regions of the gastrointestinal tract were among his most important and revealing. His findings on succession and climax of the biota in neonatal mammals and on how little diet influences the biota in adults have often been repeated by other investigators (109). His papers are landmarks in the field. Unfortunately, his work was cut short by an untimely death.

Conclusions

I have summarized in Table 3 how these investigators of the 1960s contributed to modern knowledge about the human indigenous intestinal microbiota. Each of them and their co-workers made important contributions to knowledge in the field. Their most enduring contribution, however, may be that they set in motion an explosion of research in the area that continued and expanded in the decades following the 1960s. That explosion not only enlarged our understanding of the microbiota but also gave us new perspective on how animals should be viewed as biological organisms. Animals such as humans should now be regarded as composites of animal cells and microorganisms, with the microbiota being just as important for ordinary life as the animal cells. Humans and other animals have evolved in a microbial world. Some of the microorganisms in that world have evolved into a microbiota that colonizes the intestine and has assumed certain biological functions that are essential to the life of the animal host. That microbiota has attributes of an organ of the body. Human physiology and the capacity to resist disease will only be fully understood when that microbial organ is as well understood as any other organ in the body.

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Table 3. Contributions of Investigators in the 1960s to Modern Understanding of Properties of the Human Indigenous Gastrointestinal Microbial Biota

PROPERTY	INVESTIGATORS INVOLVED
The microbiota of healthy adult humans is composed of communities of anaerobic bacteria	Drasar Gorbach Haenel Mitsuoka Smith
The bacterial members of the biota can be classified in as many as 50 genera and hundreds of species in the domain Bacteria. Some produce methane and are classified In the domain Archaea.	Mitsuoka See Text
The distal small and large intestine contains communities comprised of bacterial species present in the largest variety of genera and species	Drasar Gorbach Smith
Transient bacteria deriving from ingesta and habitats above the gastrointestinal tract can be cultured from feces and from gastric and intestinal content.	Dubos and Schaedler
The combined populations in the intestinal communities can be enormous, exceeding one hundred trillion bacterial cells	Drasar Gorbach Mitsuoka
This enormous bacterial mass forms about one-half of the fecal mass	See Text
The communities establish in the neonate in a pattern that can be recognized as an ecological succession	Dubos and Schaedler Raibaud and Ducluzeau Smith
Some species form communities in the intestinal mucous gel lining the epithelium and in the lumen	Dubos and Schaedler Gustafsson and Midtvedt
All members of the gastrointestinal communities obey ecological laws governing biological behavior and are regulated by many complex factors including some exerted by the host and some they exert themselves	Drasar Dubos and Schaedler Gorbach Gustafsson and Midtvedt Mitsuoka Raibaud and Ducluzeau Smith
The combined bacterial population, the microbiota, can be viewed as an organ of the body that is indispensable for life	Dubos and Schaedler Gustafsson and Midtvedt Mitsuoka Raibaud and Ducluzeau

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