Advances in Experimental Medicine and Biology 874
Microbial Endocrinology

Mark Lyte *Editor*

Microbial Endocrinology: Interkingdom Signaling in Infectious Disease and Health

Second Edition

Springer

Advances in Experimental Medicine and Biology

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Second Edition

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ISSN 0065-2598 ISSN 2214-8019 (electronic)
Advances in Experimental Medicine and Biology
ISBN 978-3-319-20214-3 ISBN 978-3-319-20215-0 (eBook)
DOI 10.1007/978-3-319-20215-0

Library of Congress Control Number: 2015950463

Springer Cham Heidelberg New York Dordrecht London

Printed on acid-free paper

Springer International Publishing AG Switzerland is part of Springer Science+Business Media (www.springer.com)

As always I wish to thank my family
for all their support and understanding
and especially to the Wah-wah for all his
dedication and assistance.

Preface

In the concluding section of the first chapter of the previous edition, I had written "And finally, as the oft-used cliché goes 'this is not the end of the story, just the beginning (Lyte 2010)'". The recent advances made over the last 5 years since the publication of the first edition have provided ample evidence that this has been, and hopefully will continue to be, the case. This second edition contains over 50 % new and revised content. Prominent among these has been the emergence of the microbiota–gut–brain axis and the role it plays in brain function. Microbial endocrinology, and the production of neurochemicals that the microbiota produce, provides for a mechanism (and surely *not* the sole one) by which the microbiota may influence the nervous system. In large measure, the concept of microbial endocrinology has been viewed by others as a "one-way street" in that it was usually the host's production of neurochemicals (and in the main stress-related ones) that formed the early basis for the development of the theory. The realization that metabolite production by microbiota has now been shown by groups to include many of the biogenic amines (such as the catecholamines, serotonin, and histamine) all of which are produced in quantities sufficient to impact host neurophysiology (Asano et al. 2012; Sridharan et al. 2014). As such, the other direction of this "two-way" street that has always been part of the microbial endocrinology theory to include effects of the microbiota on the host due to microbiota-derived neurochemical production is now beginning to be explored. The next few years will be, as they are also wont to say, "highly interesting". The journey continues …

Ames, IA, USA — Mark Lyte
September, 2015

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Chapter 1
Microbial Endocrinology: An Ongoing Personal Journey

Mark Lyte

Abstract The development of microbial endocrinology is covered from a decidedly personal perspective. Specific focus is given to the role of microbial endocrinology in the evolutionary symbiosis between man and microbe as it relates to both health and disease. Since the first edition of this book series 5 years ago, the role of microbial endocrinology in the microbiota-gut-brain axis is additionally discussed. Future avenues of research are suggested.

Keywords Neurochemicals • Stress • Neurophysiology • Microbiota-gut-brain • Probiotics • Microbiology

1.1 Introduction

The development of the field of microbial endocrinology has now spanned 23 years from the time I first proposed its creation in 1992 (Lyte 1992; Lyte and Ernst 1992). During that time, this interdisciplinary field has experienced two of the characteristics of a typical microbial growth curve: a long lag phase during which acceptance of articles was problematic to say the least, followed by an early log phase of growth characterized by increasing awareness that the intersection of microbiology, endocrinology and neurophysiology offers a unique way to understand the mechanisms underlying health and disease. This book, I am happy to report, comes at the start of that early log phase with the possibilities for future rapid growth.

As is more common than many are often wont to admit, the development of a new discipline does not occur in a vacuum and if one chooses to look hard enough, one can usually find reports dating back many decades, which document many of the experimental findings that help form the founding tenets of the new discipline.

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M. Lyte (ed.), *Microbial Endocrinology: Interkingdom Signaling in Infectious Disease and Health*, Advances in Experimental Medicine and Biology 874,
DOI 10.1007/978-3-319-20215-0_1

Given the history of use of neuroactive substances in the treatment of human disease, which provided for ample opportunity for the interaction of microorganisms with neuroendocrine hormones, it is not surprising that such is the case in microbial endocrinology (Lyte 2004). It is a testament to how prevailing notions of what separates "us" from "them" can influence scientific inquiry that scientists of a bygone era did not fully recognize that the ability of a lowly bacterium to both produce and recognize substances that are more commonly thought of as defining a mammal (i.e. vertebrate nervous system) could prove critical to both health and disease. Indeed, J.A. Shapiro may have put it best by titling, in part, his article covering his nearly 40-year career observing the unique growth patterns of bacteria: "Bacteria are small, not stupid" (Shapiro 2007).

1.2 From Psychoneuroimmunology to Microbial Endocrinology

1.2.1 Theoretical Reflections

During the late 1980s to the early 1990s, I, as well as many others, were involved in the examination of the ability of stress to affect immune responsiveness (Peterson et al. 1991). The field of Psychoneuroimmunology (PNI), founded by Robert Ader and Nicholas Cohen in 1975 (Ader et al. 1995; Ader and Cohen 1975), was just emerging from its infancy into mainstream thinking. That most ambiguous of biological terms, stress, was taking center stage not only in scientific thought but also in the public's perception of immediate, potentially controllable, factors that determined health and well-being. Both in the scientific and public spheres, stress has for many decades been negatively associated with health in general. The demonstration that psychological stress could impact the generation of an immune response (Ader et al. 1995), coupled with reports which showed neural innervation of immune organs such as the spleen (Felten et al. 1990), led to the realization that two seemingly disparate disciplines, one immune and one neural, interacted with each other and that interaction was critical in homeostasis and disease. While the need for such interdisciplinary research is well-recognized today as intrinsic to the study of health (witness the priority of interdisciplinary funding initiatives from the National Institutes of Health), the obviousness of such an approach was less evident in the 1970s to the early 1990s. I can well recall at conferences heated discussions from leaders in the immunology field arguing against the inclusion of neural or endocrinological factors (and certainly not something like psychological which was considered not as scientifically rigorous as a "hard" science) in the study of immune responsiveness. The advent, and increasingly accessibility, of molecular biological tools was beginning to make inroads into deciphering the mechanisms governing the generation of an immune response. Whereas immunological pathways had in the past been deciphered through the study of cell to cell interactions, molecular biological tools afforded a new way to examine such pathways and cellular

immunology began to yield to molecular immunology (with the attendant changes in departmental names). The argument by many of these immunological leaders was that with these new tools we were just beginning to understand the complexity of the immune system and to add onto that the complexity of the neural and endocrinological systems, let alone the even more unknowable psychological factors, would be scientifically "unwise" (actually, more descriptive terms were used at the time) and impede progress. Once we understood the mechanisms governing the immune system, as was the mainstream consensus at the time, only then should we tackle any interactions between different disciplines.

The recounting of the beginnings of PNI is relative to the origins of microbial endocrinology for a number of reasons. First, and foremost, the realization that an interdisciplinary approach was needed if a fuller understanding of the mechanisms that govern immune responsiveness in the host was to be achieved. That no one biological system operates in isolation of another may, on the face of it, be self-evident today; such was not the case even a quarter of a century ago. Since immunological phenomena, such as the production of antibodies, could occur in a completely *in vitro* setting (e.g. Mishell–Dutton culture) where no brain or endocrinological organs are present, why should the products of such systems, i.e. neuroendocrine hormones, be needed for an immune response? Thus, the predominant reasoning was that the immune system was a free-standing biological system that could operate in the absence of any other system. The recognition of neuroimmune interactions as being critical to the development and maintenance of immune responsiveness in an individual can best be seen in the emergence of PNI and the associated neuroimmunology-related field over the past two decades (Irwin 2008).

In many ways, microbial endocrinology has gone, and continues to go through, similar growing pains as that experienced by PNI. Cannot bacteria grow and be studied *in vitro* in the absence of any nervous or endocrinological components? Is such a question no different contextually from that which immunologists once asked of the relevance of neurohormones to the study of immunology? One of the "dirty little secrets" of the time in immunology was that the ability to demonstrate *in vitro* immunological phenomena, such as the generation of antibodies in a Mishell–Dutton system, and hence the independence of immunology from other biological systems, was that multiple lots of a key media component needed to be first screened to find the one "magical" lot that worked best. Once that lot was identified, multi-liter shipments would be ordered and stored for future use. That key media ingredient was fetal bovine serum, itself a rich compendium of neuroendocrine hormones. The realization that endocrine components were necessary to even immunological phenomena, such as antibody formation, underscored the need to study the role of such neuroendocrine influences in the individual.

That the implications of such a connection between media components and sustainability of a biological reaction was not fully recognized at the time is immediately applicable to microbiology and is best illustrated by the response engendered the first time the microbial endocrinology concept was presented at a scientific meeting. At the 1992 American Society of Microbiology 92nd General Meeting in New Orleans, I gave a 10 min slide presentation entitled "Modulation of gram-negative

bacterial growth by catecholamines" (Lyte and Freestone 2009; Mullard 2009). By the time I presented as last speaker in the session, there were only two people in the audience and the two session chairs, one of which was a well-known chair of a large microbiology department. After speaking for about 2 min about the presence of neuroendocrine hormones in bacteria and the need for an interdisciplinary approach to understanding the pathogenesis of infectious disease, one of the audience members left leaving only a solitary person in a room meant for a few hundred people. That audience member happened to be my laboratory technician, Sharon Ernst, who was a co-author on my second microbial endocrinology-related paper. At the finish of my talk, one of the chairs (not knowing I was lecturing to my own technician), evidently felt duty bound due to the presence of an audience member to ask a question, which (to paraphrase) was "why would anyone want to grow bacteria in a serum containing medium containing hormones when such good rich media exist such as tryptic soy broth and brain heart infusion". My answer (again paraphrasing from memory) was simple and still encapsulates one of the underlying tenets that have driven the creation of microbial endocrinology: "… because we do not have tryptic soy broth and brain heart infusion media floating through our veins and arteries and until we use media that reflects the same environment that bacteria must survive in, then we will never fully understand the mechanisms underlying the ability of infectious agents to cause disease".

1.2.2 Experimental Observations Leading to Microbial Endocrinology

The involvement of PNI in the creation of microbial endocrinology went far beyond the theoretical aspects described above. By 1992 I had obtained my first NIH grant which embodied a PNI approach examining the mechanisms by which stress could affect susceptibility to infectious disease. Although stress had been well recognized to affect susceptibility to infections for nearly 100 years (Peterson et al. 1991), I sought to identify relevant immune-based mechanisms through the use of the ethologically-relevant stress of social conflict (Fig. 1.1), instead of the more artificial stressors such as restraint stress or electric shock, which did not reflect any sort of stress that an animal would have any evolutionary experience (Miczek et al. 2001). Among my early findings was that social conflict stress induced an *increase* in those immune functions, notably phagocytosis, that are a first-line against infection (Lyte et al. 1990b). From an evolutionary perspective, the finding of increased immune responsiveness against infection made perfect sense. If an animal is wounded, then bacterial infection would almost certainly be encountered. It made little sense from the animal's perspective to have immune responsiveness decreased at a time that it was presented with an infectious challenge to its survival. What would be needed during this time of acute stress would be heightened immune activity which was what the social conflict study had shown.

Fig. 1.1 Social conflict in mice is conducted by the simple placement of a group-housed mouse also known as an "intruder" (in picture, *black*) into the cage of a singly-housed mouse, also known as the "resident" (in picture, *white*). The resident will engage the intruder ultimately resulting in the "defeat" of the intruder as shown by the limp forepaws and angled ears. Once the intruder assumes the defeat posture, the resident then disengages and at this point the intruder is removed. The social conflict procedure is done under reversed day-night light cycle using low level red light for illumination. For a fuller description of social conflict procedure see Lyte et al. (1990b) and Miczek et al. (2001). The social conflict procedure is done under reversed day-night light cycle using low level red light

However, this surprising result presented a paradox. If immune responsiveness is increased during time of acute, ethologically-relevant stress, then why is the animal more susceptible to an infectious challenge? Most of the literature over the last century had indeed shown that stressed animals did exhibit increased susceptibility to infectious disease challenge (Peterson et al. 1991). With that in mind, I conducted a series of experiments in 1991–1992 in which social conflict stressed animals were challenged with oral pathogens such as *Yersinia enterocolitica*. The results of those experiments showed the surprising result of increased mortality in stressed animals as compared to home cage controls (Fig. 1.2). Shouldn't these animals which showed greater than a 500 % increase in phagocytic capacity (Lyte et al. 1990a, b) also display increased resistance to infectious challenge and not the increased mortality (Fig. 1.2)?

It was these sets of experiments that in 1991–1992 led me to re-consider the whole concept of stress and susceptibility to infectious disease not from the perspective of the animal, but from that of the infecting bacterium. For a number of reasons, the infecting organism is as highly stressed, if not more so, than the stressed host. First, most infectious agents, such as food-borne pathogens have survived food preservation and cooking steps that result in a damaged cellular state. Upon entrance into

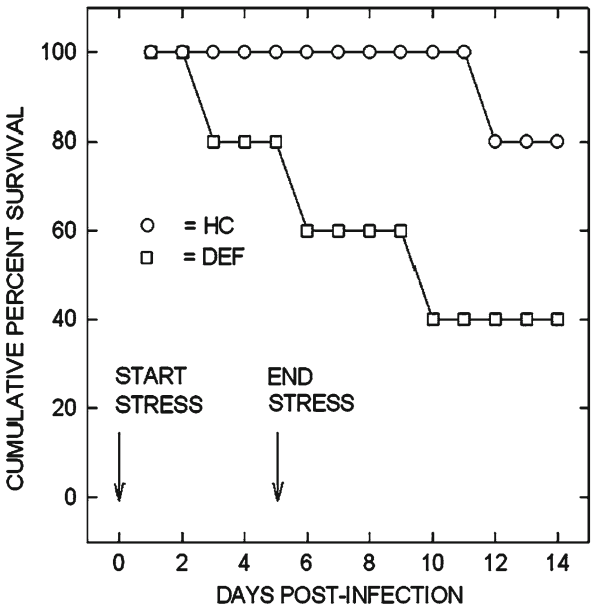

Fig. 1.2 Animals were per orally challenged with *Y. enterocolitica* immediately prior to social conflict stress (DEF, defeated, *squares*) or only handling and transport into procedure room (HC, home cage controls, *circles*). The stress or handling was conducted once per day for 5 days and percent survival followed for 14 days

the host, the infecting bacterium must survive the host's physical defenses such as stomach acid and then survive and proliferate within the gastrointestinal tract amid the trillions of indigenous bacteria which rigorously maintain ecological balance among various species through means including, for example, the elaboration of bacteriocins (Riley and Wertz 2002). Central among the factors that influence the ability of any infecting microbe to survive in a host is the capacity to recognize its environment and then employ that information to initiate pathogenic processes (i.e. adherence onto epithelium) and proliferate. The central question then became, what host-derived signals would be available to an infecting bacterium that could be used to the bacterium's own advantage and ultimately survival within the host? It was at this point that I made the decision to eliminate (for the time being) the role of immunology in addressing the effect of stress on the pathogenesis of infectious disease and instead to concentrate on the role of stress on the infecting bacterium within the hostile environment of the host. In other words, were there direct effects of the *host's* stress response on the bacterium?

Critical to the above line of reasoning was an overlooked phenomena of infectious disease as experienced in nature (real-world) as opposed to the laboratory. That aspect specifically concerns the dose of infectious organisms that are needed to effect overt disease in the host. It is well established in food microbiology that the number of infecting organisms needed to cause food-related gastrointestinal infection can be as low as 10 bacteria per gram of food (Willshaw et al. 1994). However, in the laboratory, the challenge of animals with infectious bacteria can well go as high as $10^{10–11}$ bacteria or colony forming units (CFU) per mL. Further adding to

this discrepancy between real-world and laboratory infectious doses, is that on average a mouse weighs 20–25 g while, on average a human weights 70 kg individual, meaning that the dosage a laboratory animal receives is many-fold greater than what is experienced by an individual. Over the last century a number of investigators have raised the issue of whether non-ecologically relevant doses of infectious organisms can provide complete understanding of the mechanisms that underlie the pathogenesis of infectious disease *in vivo* (Smith 1996). In a similar fashion, this same question can also be raised regarding *in vitro* studies which utilize high ($>10^4$ CFU per mL) bacterial inoculums. Not unlike the question of how a *single* individual may respond to a new environment as compared to how a large *group* of individuals may respond to the same new environment, the survival behavior of low numbers of bacteria within the new environment of the gastrointestinal tract may radically differ from that of large numbers of bacteria. This social aspect of bacterial behavior represents the newly emerging field of sociomicrobiology (Parsek and Greenberg 2005; West et al. 2006). Specifically, the environmental signals that *single* or low numbers of bacteria may look for markedly differ from that sought by high numbers of bacteria. And in addition to the above point of low, not high, numbers of bacteria which contaminate food, this also applies to the vast majority of infections in general in which infecting doses of bacteria are small ($<10^4$ CFU) in number.

Thus, from the outset one of the guiding principles in microbial endocrinology has been the use of low bacterial numbers (1–10^3 CFU per mL) coupled with a medium that is reflective of the *in vivo* milieu. Other guiding principles, such as the combination of neuroendocrine hormones and bacteria under study should be matched such that each is found to occur in the same anatomical region *in vivo*, have also been formulated. In addition to the chapters contained in the present book, the reader is further directed to a comprehensive review which thoroughly discusses the methodological aspects of conducting microbial endocrinology-related experiments (Freestone and Lyte 2008).

The choice of the initial neuroendocrine hormones for the first experiment was based on the stress response itself and the well-known increase in catecholamines (Woolf et al. 1992; Gruchow 1979). Further, the stress-induced release of catecholamines had been one of the primary mechanisms that had been proposed in PNI-related research to account for the ability of stress to suppress immune responsiveness and hence increase susceptibility to an infectious challenge (Ader and Cohen 1993; Webster Marketon and Glaser 2008). As has been recognized for many decades, the induction and sustained release of the catecholamines, especially norepinephrine, occurs during many forms of stress extending from psychological to surgical (Fink 2000). The Gram-negative bacterium, *Y. enterocolitica* was chosen as the first bacterium to test whether a neuroendocrine hormone, namely norepinephrine, could have *direct* effects on growth. The results of this initial experiment in 1992, which was carried out in liquid culture using small 60 mm Petri dishes, combined a low inoculum of *Y. enterocolitica* (33 CFU per mL of serum-supplemented SAPI) with norepinephrine, epinephrine or diluent (Fig. 1.3). In many ways this experiment, which was the proverbial "shot in the dark", is the one that has led through the many years to the creation of this current book. As shown in Fig. 1.3, there is a very small

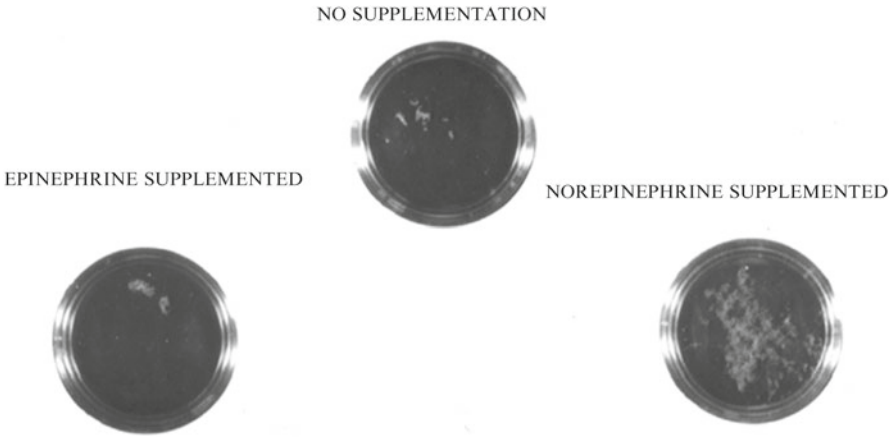

Fig. 1.3 The experiment that launched the field of microbial endocrinology. *Yersinia enterocolitica* culture plates in 1991 showing that bacterial growth in serum-based medium was enhanced in the presence of the neuroendocrine stress hormone norepinephrine, but not epinephrine or control diluent

amount of visual growth evident in both the control and epinephrine supplemented plates (indicated by arrows). However, in the norepinephrine supplemented culture, there is dense growth throughout. To this day I still remember my excitement at seeing these results. And from that day on, I effectively ceased looking at PNI-related phenomena and instead turned my research direction to the study of neuroendocrine-bacterial interactions and the creation of the field of microbial endocrinology.

1.2.3 Gaining Acceptance of Microbial Endocrinology

As can often be the case in any endeavor which seeks to introduce a paradigm-shift in thinking, the introduction of neuroendocrine-bacterial interactions as a hitherto unrecognized mechanism in the pathogenesis of infectious disease was met not only with initial skepticism, but also downright hostility. At a mid-1990s meeting in Toronto that focused on the role of neuroendocrine mediators and immunity in drug addiction, I gave a microbial endocrinology-based lecture as part of a session on stress and its relationship to drug addiction sequelae such as increased prevalence of infectious disease in drug addicts. At the conclusion of my talk before I could take any questions, the session chair addressed the audience and said that my ideas were so radical that they should not be taken seriously and the audience should in essence forget what I just presented. More than one member of that audience has approached me over the years to recount that episode and the shock of the audience being told to disregard what they had just heard as well to ask why I didn't get mad (which I didn't). Such opposition, although admittedly more restrained, was also

encountered during the early years in terms of gaining acceptance into the scientific literature. I have been told by more than one individual that the integration of microbial endocrinology into mainstream infectious disease research would have been accelerated if I had chosen to publish in more microbiology-oriented journals. However, my choice to publish in journals not typically read by microbiologists was dictated not by choice, but instead by necessity. My early attempts to publish in basic microbiology-based journals were universally met with rejection. Undoubtedly, while one may take the convenient rode of blaming the reviewer for failure to consider a highly interdisciplinary approach where it is often not possible to address all the questions regarding each of the fields, I shall instead take a fair share of the blame since it is also the responsibility of the author to educate the reader of the need to go beyond traditional thinking.

With that said, I have also come to recognize that one of the defining reasons that these early papers were rejected from basic microbiology-centric journals was the reliance on phenomenology rather than mechanisms. My own training in the clinical laboratory sciences and subsequent work in hospital laboratories before entering graduate school in 1977, ingrained in me a powerful sense of the clinical side of microbiology. And that side is one that is grounded in growth for without evident growth and sufficient numbers of bacteria little can be done, even today, to diagnosis suspected bacterial disease. Thus, it seemed to me at the time (and still does today) that the ability to show growth-related effects of neuroendocrine hormones on bacteria would have profound implications for the study of the host factors which influence susceptibility to infectious disease. However, I was surprised that this was generally not the case. A similar refrain ran through those early reviews that the demonstration of effects on growth were phenomenological in nature and what needed to be shown was the mechanism(s) by which neuroendocrine hormones could influence bacterial physiology. Due largely to the availability of an ever growing arsenal of molecular biological techniques, phenomenology was to be eschewed in favor of dissecting molecular mechanisms. While I do not mean to begrudge nor demean the value of mechanistic studies, one may argue that many of the advances in the treatment of disease have been made through the observation of phenomena for which no mechanism at the time of discovery was available. Antibiotic development owes itself largely to the observation of phenomena. While the requirement for molecular analyses currently reigns dominant in the majority of first-tier microbiology journals, the relegation of phenomenological studies to the status of second-class research ignores its historically pivotal role in fueling scientific and medical advances. A number of articles examining the failure of genomic-based strategies to lead to the discovery of new antimicrobials that ultimately make the transition from the lab bench to clinic have addressed this very point (Finch 2007; Barrett 2005).

My reasoning for discussing the relative merits of phenomenology versus molecular analyses is not to point out my own shortcomings in the area of molecular analysis, but instead to offer a cautionary note to other researchers who may choose to explore microbial endocrinology. Catecholamines, which to date have been the principal neuroendocrine hormones that have been examined in the microbial

endocrinology field by virtue of their prominence in the stress response, represent but a tiny sliver of the spectrum of neuroendocrine hormones that can be examined for potential interaction with both pathogenic as well as commensal bacteria. For example, gamma amino butyric acid (GABA), the primary inhibitory neurotransmitter in the mammalian brain, is produced in such large amounts by bacteria in the gut that a role for bacterial-derived GABA has been proposed to account for the altered brain function (encephalopathy) that is part of the pathogenesis of advanced liver disease and sepsis (Minuk 1986; Winder et al. 1988). In fact, GABA produced by bacteria, such as those contaminating a distilled water apparatus, have been found not only to confound neurotransmitter binding studies with mammalian cells (Balcar 1990), but also to possess a high affinity binding protein that resulted in one of the first bioassays for GABA that was entirely bacterial-based (Guthrie and Nicholson-Guthrie 1989; Guthrie et al. 2000). In this book, Chap. 4 by Victoria Roshchina provides an exhaustive review of the wide breadth of neurohormones that are found in prokaryotes that we otherwise only associate with multi-cellular eukaryotic systems.

By utilizing a microbial endocrinology approach, researchers can further our understanding of how host and bacteria, both commensal and pathogenic, interact in the gut (or at other sites). That approach, in turn, could provide insights into not only homeostasis but also other medical conditions that involve gut pathology that upon verification could enable the design of new innovative medical interventions. Although researchers realized more than 100 years ago that the mammalian gut is innervated, how this system interacts with the gut microbial flora remains largely a mystery. Further, large amounts of neurochemicals are produced within the gut that find its way into the gut lumen where the possibility of interactions with the gut microflora exist and remain largely unexplored. For example, large quantities of serotonin are produced by the gut that can be recovered from the lumen, although the physiological reason for this production are not well understood. Could it be that serotonin produced by the mammalian gut has some hitherto unknown interaction with a specific part of microbial population? Thus, examination of any such serotonin-bacterial interaction will depend on both *phenomenology* and molecular analyses to provide as complete a picture as possible of the relevance of microbial endocrinology to both homeostasis and disease.

Further, the bi-directional nature of bacterial-microbial interactions contained with the theory of microbial endocrinology also suggests that bacteria can influence mammalian function. Work utilizing metabolomics to compare the blood metabolic profile of conventional-reared and germ-free mice revealed that the gut microbiome contributed to the concentration of neuroactive components in the circulation (Wikoff et al. 2009). That the presence of a microbial community within the gut, and inherent interactions between the host and gut microflora, is crucial to an animal's neurological health was demonstrated in 2004 when Nobuyuki Sudo and colleagues at Kyushu University in Japan examined the role of microbial colonization on the hypothalamic-pituitary-adrenal response to stress in gnotobiotic, germ-free and conventionally-reared mice (Sudo et al. 2004). Not only did the development of host neural systems that control the physiological response to stress depend on

postnatal microbial colonization of the gut, but reconstitution of gnotobiotic mice with feces from specific pathogen-free mice altered their subsequent neurohormonal stress response. Additionally, Li et al. demonstrated that diet-induced alteration of gut microbial diversity can even affect memory and learning in mice (Li et al. 2009). Thus we are just beginning to understand the degree to which microbial diversity is crucial to the development and regulation of normal gastrointestinal function. Does gut neuronal activity influence local bacterial ecology and vice versa?

1.3 Collaboration and Dissemination

The development of any field is often dependent on the interactions and potential collaborations with others. Since the initial first sole authored reports in which the concept of *direct* microbial endocrinology-based interactions (Lyte 1992, 1993) was reported and the theory of its proposed role in health and disease was discussed, it has been the subsequent efforts of graduate students, technicians and fellow scientists that has been instrumental in the growth of microbial endocrinology. While over the course of time some of these collaborations have remained strong, for example, graduate students some of whom later became collaborators in the examination of microbial endocrinology in the microbiota-gut-brain axis (Galley et al. 2014; Lyte et al. 1998), others ended often after running their natural course while others came to a more acrimonious ending. As this chapter is titled, in part, "a personal journey", the realization that one highly productive collaboration had abruptly ended occurred one late afternoon after a literature search just by happenstance referenced a document in a European data that upon reading, and seeing mutual experimental ideas discussed but no mention that it was indeed collaborative, felt like stepping off a curb, turning to one's side, and getting hit by a high speed truck. The pursuit of science is not often the straight and collegiate course one imagines as a student.

A critical point in the development of microbial endocrinology turned out to be a fortuitous meeting at the 1995 First International Rushmore Conference on Mechanisms in the Pathogenesis of Enteric Diseases held in Rapid City, South Dakota. Following my presentation, I was approached by a bearded and pony-tailed Richard Haigh, at the time a Ph.D. student at Leicester University in the United Kingdom. Richard's interest in my work served as the bridge to Primrose Freestone who was a post-doctoral fellow in his lab in the then Department of Microbiology and Immunology within the medical school. During this time I also consider myself fortunate to have helped interest other investigators to examine bacterial-neuroendocrine interactions. For example, during microbiology meetings both in the United States and in Japan at which I and my colleagues had presentations, I was approached by James Kaper of the University of Maryland and his then postdoctoral fellows Jorge Girón and Vanessa Sperandio. Following stimulating conversations with them regarding my concept of bacteria recognizing hormones and the potential