

Microbial Endocrinology Comes of Age

Microorganisms interact with host endocrine systems, augmenting disease processes and possibly playing a role in development

Mark Lyte and Primrose Freestone

Microbial endocrinology is the discipline where microbiology, endocrinology, and neurophysiology intersect. Its objective is to examine and better understand how microorganisms interact with their hosts under circumstances of both health and disease. A fundamental tenet is that microorganisms carry the means for sensing host hormones, which provide environmental cues that trigger growth and may lead to pathogenic processes.

Indeed, bacteria respond to mammalian hormones signals as environmental cues—an evolutionary development that should not come as a surprise. For one thing, neuroendocrine hormones are not confined to vertebrates but are

widely distributed in nature. For example, the neuroendocrine hormones norepinephrine and dopamine also are found among diverse other species, including bananas, potatoes, tomatoes, insects, and protozoa. Similarly, corticotropin can be isolated from *Tetrahymena pyriformis*, while *Pseudomonas fluorescens* produces a high-affinity receptor for γ -amino butyric acid (GABA), an inhibitory neurotransmitter from the mammalian central nervous system. This wide distribution of neuroendocrine hormones across nature means that microorganisms have had ample opportunity to encounter, recognize, and respond to these molecules as environmental signals, including by changing growth patterns and producing virulence factors.

Summary

- Some bacterial pathogens directly respond to stress-induced neuroendocrine hormones, reacting to stress in parallel to host immune cells.
- When catecholaminergic nerves within the gut release norepinephrine, the local population of gram-negative bacteria, mainly *Escherichia coli*, increases by as much as 10^5 -fold.
- Because catecholamines enhance bacterial attachment to plastics and thereby biofilm formation, we should consider such drug side effects when treating patients in intensive care units or similar settings.
- Stress hormones not only enhance growth, but also modulate expression of virulence-associated genes in some bacterial pathogens.
- Because bacteria both produce and respond to neuroendocrine hormones, understanding how these factors influence particular pathogens could lead to new ways of preventing infectious diseases.

Views over How Stress Affects Infections Change with the Centuries

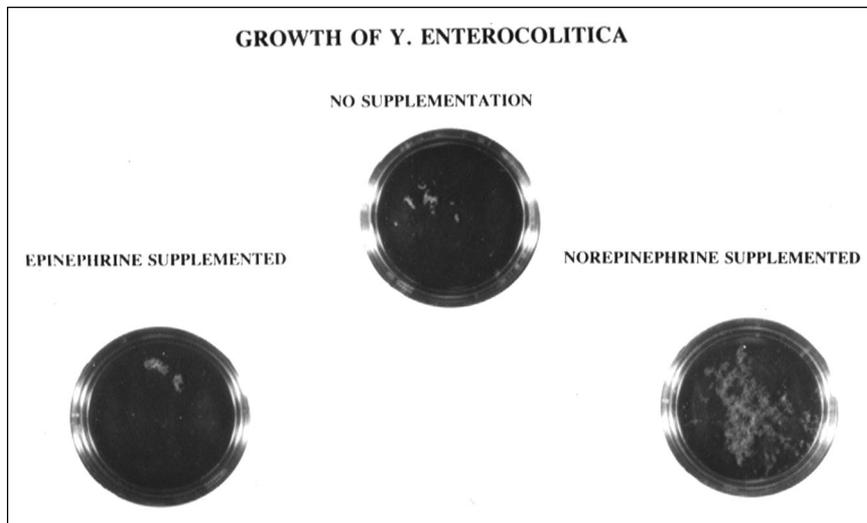
In the third century A.D., the physician Galen recognized that negative emotions such as melancholy, now recognized as stress, predisposed women to develop “ill humours” (see box, p. 174). However, another 1,600 years elapsed before physicians and scientists began to understand why stress could affect susceptibility to illnesses, including infectious diseases.

Stress-related neuroendocrine hormones, specifically the catecholamines, directly influence immune functions. Data supporting this link persuaded mainstream immunologists that the immune system is not free standing, but instead interacts with neuroendocrine components and products. At first, the main focus was on how stress modifies immune responses, typically by suppressing them. Thus, immunologists and

Mark Lyte is a professor in the Department of Pharmacy Practice, School of Pharmacy, Texas Tech University Health Sciences Center, Lubbock, and Primrose Freestone is a lecturer in clinical microbiology in the Department of Infection, Immunity and Inflammation, University of Leicester School of Medicine, Leicester, United Kingdom.



FIGURE 1



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 (see text for more explanation for a description of these foundation experiments).

others generally accepted the idea that stress can increase an individual's susceptibility to infectious challenges. However, few experts stopped to think whether infectious microorganisms were active participants in these processes, except to acknowledge that pathogens proliferate when stress compromises the host's immune responses.

However, microorganisms are not mere bystanders. Indeed, some pathogens directly respond to stress-induced neuroendocrine hormones, reacting to stress in parallel to host immune cells. Evidence supporting this view has been around for a long time. For example, in 1930, two French clinicians, M. Renaud and A. Miget, reported that an individual with urticaria developed fulminant gas gangrene only six hours after being injected with epinephrine. They determined that the syringe was contaminated through use with another patient already suffering from gas gangrene. However, the speed with which the clostridial infection developed was particularly intriguing.

Similar reports linking epinephrine injections with clostridial infections also appeared. Indeed, 70 years later, it remains standard practice to coadminister epinephrine and *Clostridium*

perfringens to mice or other rodents to facilitate the development of gangrene when studying this condition. Researchers invariably explained this enhancement of infection as due to the suppressive effects of this hormone on host immune responses. However, this one-sided idea began to shift when one of us (M.L.) reported in 1992 that some bacteria directly respond to stress-related host signals. By 1999 we had demonstrated that recognition of stress hormones was widespread in the bacterial world.

Where Mammalian and Microbial Worlds Collide: the Gut

The enteric nervous system (ENS) runs the entire length of the gastrointestinal tract and contains nearly 500 million neurons, as many as are in the spinal cord. A part of the ENS innervates the villi, which interface the gut lumen and its trillions of bacteria. Changes in nervous system activity influence bacteria within the gut and lead to major shifts in bacterial flora. For example, when we administer the neurotoxin 6-hydroxydopamine (6-OHDA) to mice, the catecholaminergic nerves within the gut (and at other anatomic sites) release norepinephrine. Within 24 hours, the population of gram-negative bacteria, mainly *Escherichia coli*, within the gut increases by as much as 10^5 , with most of those cells either in the cecal lumen or attached to the mucosal wall, which is an early stage in pathogenesis. As the catecholaminergic nerves regenerate during the following two weeks, the cecal flora returns to what it had been. Thus, changes in the neuroendocrine environment perturb the gut flora and could explain how dramatic elevations in plasma catecholamines that follow trauma, including surgery and postoperative complications, can lead to infectious disease.

Because bacteria can sense our hormones, bacteria in specific anatomic niches are likely to respond specifically to hormones that they regularly encounter. For example, compare the catecholamine responsiveness of three important pathogens, *E. coli* O157:H7, *Salmonella enterica*, and *Yersinia enterocolitica*. As a patho-

Lyte: Persuading Microbiologists that Bacteria Recognize Stress Hormones

When Mark Lyte began his talk on microbial endocrinology during the 1992 ASM General Meeting, only two people were seated in the audience, and one walked out after 2 minutes, leaving his technician, who *had* to be there. By 2007 in Toronto when he spoke on the same topic, the house was packed. “My earlier talk was the first time that the concept of microbial endocrinology was being introduced, and people thought that I was a bit off-the-wall, to say the least,” he recalls. “I could spend a long day explaining how people approach science and when ideas are ready to be accepted.”

At the ASM General Meeting in Toronto, where Lyte and Freestone convened the first symposium on microbial endocrinology, “the community was ready to embrace the concept and has come around to see that the worlds of bacteria and vertebrates can interact in a way previously not recognized,” Lyte continues. “I kept persisting at it, and have presented the concept whenever and wherever I have been given a chance to do so. I just knew I had to be right. Once you see that the addition of a tiny amount of hormone results in billions of times more bacterial growth, you know you have found something.”

However, during much of that time, Lyte felt ignored by mainstream microbiology. “One time, after I gave a talk, I sat down to lunch and one of the audience members who was at my table said to me, ‘you know what you are, don’t you? You’re the prototypical iconoclast,’” he says. “That has helped me weather a lot of storms.”

Lyte, professor in the school of pharmacy at the Texas Tech University Health Sciences Center and

director of translational medicine at the Texas Tech Garrison Institute of Aging, has never wavered. He continues to focus on the interface between microorganisms and the neuroendocrine system in both health and disease. And he envisions numerous practical applications to the work.

For example, one particularly serious infectious disease problem in intensive care units (ICUs) arises when indwelling medical devices such as catheters become contaminated with bacteria from the skin, such as *Staphylococcus epidermidis*. “Using microbial endocrinology-based principles, we showed that the drugs that flowed through the catheters were actually inducing *S. epidermidis* to grow,” he says. The drugs, inotropes, are administered to patients in ICUs to maintain cardiac and kidney function, and are neuroendocrine-based. “Thus, it is the drugs that are used to help the patient that also enable normal skin bacteria such as *S. epidermidis* to cause infections,” he says. “If you can interrupt the ability of bacteria to recognize neuroendocrine hormones and thus sense their surroundings and initiate pathogenic processes, then you can design a whole new class of antibiotics.”

Lyte, 54, grew up in Bergenfield, N.J., near New York City. He describes his childhood as “pretty normal. I participated in things like science fairs in high school, and my project did end up in my being selected as one of two students from my state to be awarded a National Aeronautics and Space Agency-National Science Teachers Association Science Award, complete with a trip to the Goddard Space Center,” he says. As a youngster, he

also enjoyed “watching movies of scientists battling the odds,” and also cites a film on the life of Paul Ehrlich entitled *The Magic Bullet* as a long-time favorite.

His undergraduate degree from Fairleigh Dickinson University in New Jersey in 1976 was in medical technology. “I was the first male ever accepted to the hospital where I did my clinical internship,” Lyte recalls. “Remember that the clinical lab was still almost exclusively female, even in the 1970s. That clinical experience, coupled with running hospital clinical labs for the next two years, gave me an appreciation of health and disease that has influenced my thinking ever since. I didn’t want to go to a typical grad school in the States, as I was very hands-on.” Instead, he left for Israel to study at the Weizmann Institute of Science in Rehovot, where he received his M.S. in 1979 and his Ph.D. in 1983.

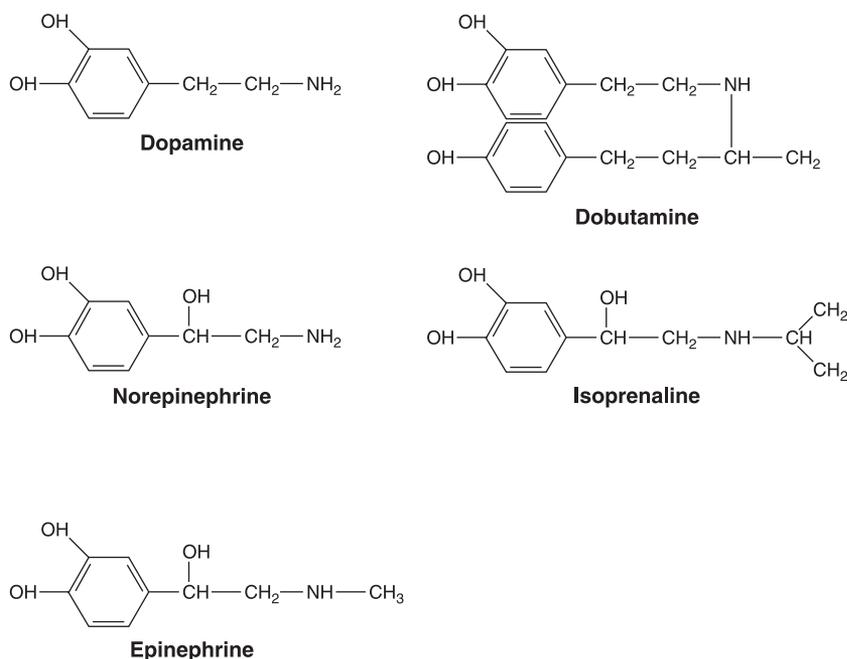
Lyte has been married for 31 years. His wife, a former classical musician, is trying to break into the writing world and has been freelancing for the local newspaper and city magazine. They have two sons, both in college. “They want to be professors someday,” he says. Lyte took a special pleasure in bringing his older son to the ASM Toronto meeting in 2007. “It was really neat for him to see me talk to such a large audience at the microbial endocrinology symposium because he always remembered my telling the family what my prior experience—at the 1992 meeting—had been,” he says.

Marlene Cimons

Marlene Cimons is a freelance writer in Bethesda, Md.



FIGURE 2



Catecholamine and inotropic agent structures. In eukaryotic systems the amino acids tyrosine and phenylalanine are the precursors for the stress-related neuroendocrine hormones dopamine, norepinephrine, and epinephrine. Dobutamine and isoprenaline are synthetic inotropic agents, used therapeutically in the treatment of cardiovascular and renal conditions.

gen, *Y. enterocolitica* primarily inhabits the gut, whereas *E. coli* O157:H7 and *S. enterica* colonize extraintestinal sites.

Meanwhile, these strains prefer norepinephrine and dopamine to epinephrine. For example, *Y. enterocolitica* isolates do not respond to epinephrine, and it antagonizes the responses of 14 *Y. enterocolitica* strains to dopamine and norepinephrine. Why? Noradrenergic and dopaminergic neurons are abundant within the nervous system, producing norepinephrine and dopamine, respectively. However, these neurons do not produce phenylethanolamine *N*-methyltransferase, the enzyme that converts norepinephrine into epinephrine, accounting for the latter's absence from the enteric nervous system. Thus, enteric microbes are missing recognition systems for this host factor, which they rarely, if ever, encounter.

In mammalian systems, catecholamine recognition typically involves specific adrenergic or dopaminergic signaling pathways and receptors. Thus, for example, antagonists that block cate-

cholamine receptors in humans are therapeutically important for treating conditions such as hypertension. Although genomic analyses reveal no adrenergic and dopaminergic receptors in bacteria, we find that adrenergic and dopaminergic antagonists block norepinephrine, epinephrine, and dopamine responsiveness in both gram-negative and -positive bacteria.

Intriguingly, only α -adrenergic antagonists, but not β -adrenergic antagonists, block bacterial responses to norepinephrine and epinephrine. Conversely, dopaminergic antagonists block responses to dopamine, but do not affect responses of bacteria to either norepinephrine or epinephrine. These findings indicate that the bacterial catecholamine response systems resemble the α , but not β , systems of mammals. Similarly, there is a bacterial response system with dopaminergic-like specificity. These findings further suggest that enteric bacteria respond specifically to catecholamines that they are likely to encounter in the gut. It will be interesting to determine the catecholamine specificity of bacterial species that occupy other anatomic sites.

Microbial Endocrinology May Affect Patient Outcomes in Intensive Care Units

Infections are a major cause of morbidity and mortality among acutely ill patients, particularly those being treated in hospital intensive care units (ICU), where infection rates typically are higher than elsewhere in hospitals. However, viewing ICU patients through the lens of microbial endocrinology may provide insights into why such patients are so susceptible to infections, despite intensive antibiotic prophylaxis.

Patients in ICUs typically receive catecholamine hormones or related compounds to maintain essential organ functions (Fig. 2). These same agents can increase the growth of pathogens that cause nosocomial infections for such patient (Fig. 3). For instance, in the presence of norepinephrine or dobutamine, the growth of nonadherent coagulase-negative staphylo-

cocci in plasma-supplemented media increases by several orders of magnitude.

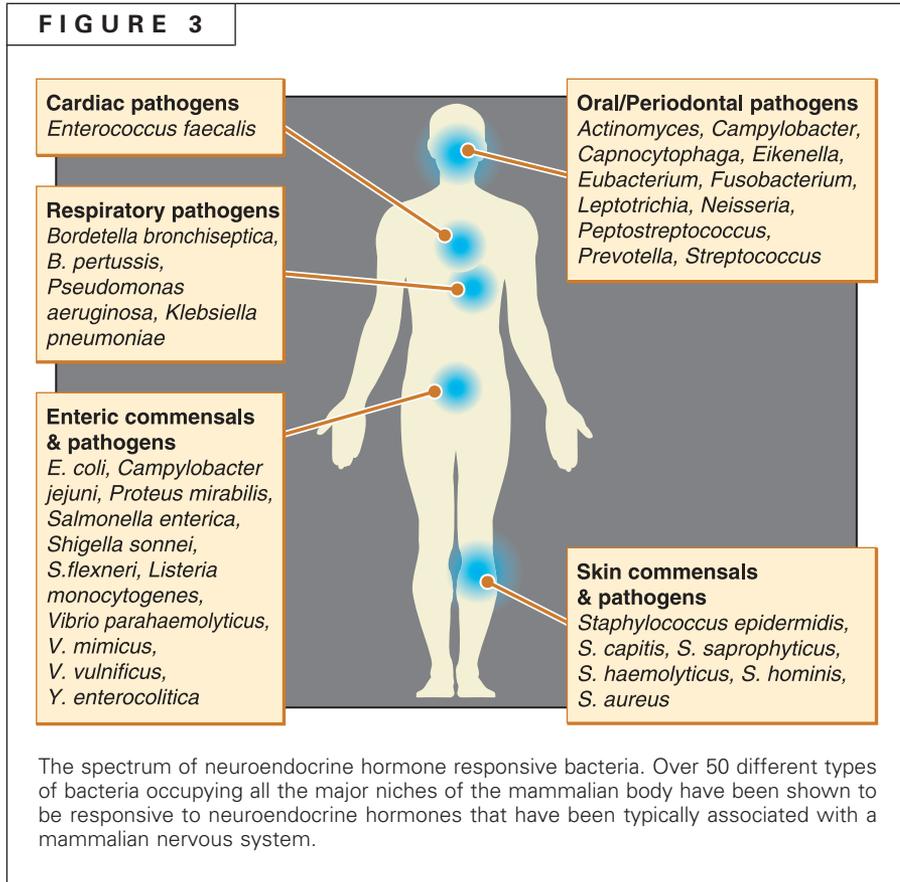
Intravascular catheters are a common source of biofilm-related infections for patients in ICUs, particularly from skin commensals such as *Staphylococcus epidermidis*. Catecholamines, in addition to accelerating planktonic growth of staphylococci, also enhance biofilm attachment to catheter-grade plastics. Further, we showed that catecholamines also facilitate the recovery of bacterial pathogens such as *S. aureus*, *S. epidermidis*, and *S. haemolyticus* following antibiotic treatment. Overall, our findings suggest that we may need to take into account drug side effects on microbial growth when treating patients in hospital ICUs or in similar settings.

Investigating Mechanisms of Bacteria-Neuroendocrine Hormone Interactions

According to our early investigations, stress hormones increase bacterial growth through two main mechanisms. In the first case, stress hormones form complexes with iron-sequestering proteins such as transferrin and lactoferrin, reducing their affinity for iron and thus allowing bacteria to obtain iron from these ordinarily inaccessible host stores.

The other mechanism is independent of host iron and, instead, involves synthesis of a novel growth stimulator among many different species of bacteria. This autoinducer restores active growth for several pathogens, including *E. coli* O157:H7 and *Salmonella enterica*, and it also induces *Bacillus anthracis* spores to germinate.

Several investigators have attempted to understand the global response of bacteria to stress hormones by looking at gene expression profiles. Interpreting these microarray analyses of enteric bacteria such as *E. coli* and *Salmonella* becomes complicated in part because of the diversity of media on which these bacteria are grown and of the varied physiological states of the cultures being profiled. Nevertheless, stress hormones not only enhance growth, but they also modulate expression of virulence-associated genes.



Bacterial interactions with catecholamine hormones reflect the role that stress plays in infectious diseases. However, other hormones also influence virulence. For instance, fungal pathogens such as *Coccidioides* and *Candida* possess receptors for estrogen, which may help to explain why women become so much more susceptible to thrush infections when they are pregnant. Moreover, estradiol directly enhances the infectivity of *Chlamydia trachomatis*.

In the case of bacterial pathogens, *Burkholderia pseudomallei* synthesizes and responds to human insulin, helping to account for this species being a scourge for individuals with insulin-dependent Type 1 diabetes. Meanwhile, quorum-sensing *Pseudomonas aeruginosa* cells become more virulent in response to the opioid peptide dynorphin from neurons. Genome analysis of *Neisseria meningitidis* reveals it contains a gene for a fully functional mammalian β -endorphin receptor, providing yet another example of microbial endocrinology yielding valuable insights into infectious agents.



Developmental Timeline of Microbial Endocrinology

A.D. 300: Galen notes emotional state (stress) contributes to human disease.

1930: Renaud and Miget report fulminating sepsis in patients given epinephrine for urticaria.

1946: Cooper reports catecholamines decrease the number of bacteria needed to cause infection in mice by 1 million-fold.

1948–55: Miles repeats and extends earlier work of Cooper. Concludes that mechanism responsible for catecholamine-induced bacterial growth must be due to action on immune cells although experimental results do not fully support such a conclusion.

1970s–80s: Reports of bacterial receptors for insulin as well as other neuroendocrine hormones appear.

1992: First report by Lyte of *direct* neuroendocrine-bacterial interactions; formulation of the theory of microbial endocrinology.

1996–2000: Mechanisms by which catecholamines induce bacterial growth begin to be elucidated.

2004: First review on microbial endocrinology.

2006: Report showing possible cross-connection between microbial endocrinology and quorum sensing.

2007: Demonstration that specificity exists in bacterial catecholamine response pathways.

2007: First symposium on microbial endocrinology at ASM General Meeting.

2009: Second symposium on microbial endocrinology at ASM General Meeting and upcoming book.

Experimental Considerations, Caveats

The environment in which bacteria are placed is crucial for their behaviors. In the laboratory, choosing media that fosters rapid growth can be a major factor determining the outcome of experiments. The question arises whether the choice of a rich medium is appropriate if the goal is to understand how bacteria behave in particular host body fluids that contain immune components, iron-restricting glycoproteins, and neuroendocrine hormones.

One defining principle for microbial endocrinology is that the more closely a medium reflects the *in vivo* environment, the more likely we will accurately observe the effects of any one neurohormone. Further, the amount of inoculum is also crucial. Thus, lower starting amounts of bacteria more closely reflect *in vivo* conditions at the time the infectious challenge occurs. We recently published a guide for designing experiments in microbial endocrinology that takes such effects into account.

Directions for Microbial Endocrinology Research

Many microbiologists now realize that bacteria not only produce, but also actively respond to neuroendocrine hormones. Understanding how these environmental factors influence particular bacterial pathogens could also suggest new ways to prevent infectious diseases. For example, small molecules block binding of catecholamines to a bacterial receptor, thereby protecting mice against *Francisella tularensis*, according to Vanessa Sperandio at the University

of Texas Southwestern Medical Center in Dallas and her collaborators.

Researchers studying how hormones and bacteria interact so far focused primarily on stress-related catecholamines, leaving a great panoply of neurohormones yet to be examined. 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. 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.

In 2004, Nobuyuki Sudo and colleagues at Kyushu University in Japan provided an important demonstration of the interactions between the host and gut flora. 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. And more recently, Wang Li and colleagues at Texas Tech showed that diet-induced alteration of gut microbial diversity can even affect memory and learning in mice. 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?

SUGGESTED READING

- Freestone, P. P., R. D. Haigh, and M. Lyte. 2008. Catecholamine inotrope resuscitation of antibiotic-damaged staphylococci and its blockade by specific receptor antagonists. *J. Infect. Dis.* **197**:1044–1052.
- Freestone, P. P., and M. Lyte. 2008. Microbial endocrinology: experimental design issues in the study of inter-kingdom signalling in infectious disease. *Adv. Appl. Microbiol.* **64**:75–105.
- Freestone, P. P., S. M. Sandrini, R. D. Haigh, and M. Lyte. 2008. Microbial endocrinology: how stress influences infection. *Trends Microbiol.* **16**: 55–64.
- Lyte, M. 1993. The role of microbial endocrinology in infectious disease. *J. Endocrinol.* **137**:343–345.
- Lyte, M. 2004. Microbial endocrinology and infectious disease in the 21st century. *Trends Microbiol.* **12**:14–20.
- Lyte, M., and M. T. Bailey. 1997. Neuroendocrine-bacterial interactions in a neurotoxin-induced model of trauma. *J. Surg. Res.* **70**:195–201.
- Lyte, M., and S. Ernst. 1992. Catecholamine induced growth of gram negative bacteria. *Life Sci.* **50**:203–212.
- Rasko, D. A., C. G. Moreira, de R. Li, N. C. Reading, J. M. Ritchie, M. K. Waldor, N. Williams, R. Taussing, S. Wei, M. Roth, D. T. Hughes, J. F. Huntley, M. W. Fina, J. R. Falck, and V. Sperandio. 2008. Targeting QseC signalling and virulence in antibiotic development. *Science* **321**:1078–1080.
- Zaborina, O., F. Lepine, G. Xiao, V. Valuckaite, Y. Chen, T. Li, M. Ciancio, A. Zaborin, E. O. Petrof, J. R. Turner, L. G. Rahme, E. Chang, and J. C. Alverdy. 2007. Dynorphin activates quorum sensing quinolone signaling in *Pseudomonas aeruginosa*. *PLoS Pathog.* **3**:e35.

Q: When does
 $4 \times \$17 = \$48?$

A: When Professors,
 Mentors and
 University Department
 Chairs purchase 4 or more
 student memberships for
 their
 undergraduate and
 graduate scholars!

Buy 4 Student Memberships and receive a discount of \$5 per membership!

To take advantage of this offer or to get more information contact Membership Services at membership@asmusa.org. You can also go to the Membership Menu to get started <http://www.asm.org/Membership/index.asp?bid+363>.



1752 N Street, NW
 Washington, DC 20036
<http://www.asm.org>
membership@asmusa.org

Postdoctoral Membership

Recognizing the unique status of scientists during the postdoctoral training phase of their careers, ASM now offers Postdoctoral Membership.

Now postdoctoral trainees can receive all the privileges* of Full Membership for \$37 a year, for 4 consecutive years – two years longer than the Transitional Member rate offers.

The 2007 membership fee will be \$37 (\$35 for those who join or renew online). Any microbiologist who has earned a doctorate within the past 12 months is eligible. The Postdoctoral rate may not be combined with the Transitional rate to allow 6 years of reduced rate membership.

Contact Membership Services at service@asmusa.org for more information, or join online at estore.asm.org today!



1752 N Street, NW
 Washington, DC 20036
<http://www.asm.org>
service@asmusa.org

*except the right to vote or hold office

Microbial endocrinology: The interplay between the microbiota and the endocrine system. Neuman H. Debelius J. Knight R. et al. See more. FEMS Microbiology Reviews. Future research in this area will reveal additional connections, and elucidate the pathways and consequences of bacterial interactions with the host endocrine system. Author supplied keywords. Endocrine system. Germfree. Hormones. Immunity. 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. Download full-text PDF. Source.