ETSU

January 2013

DBMS – News & Events

A Newsletter of the Department of Biomedical Sciences

Dr. Gregory Ordway, Interim Chair
Department of Biomedical Sciences

It was a delight to see such a great turnout for the DBMS Holiday gathering at the Carnegie. We hope that this will be an annual event, as it allows us in a small way to show our appreciation of all the dedicated employees of the Department.

With this upcoming transitional year, one of the major goals is to strengthen our research and teaching through the recruitment of new faculty. Many thanks to all of those involved in the recruitment process. Please be sure to take part in the process by speaking with committee members.

"Coming together is a beginning, Keeping together is progress. Working together is success." -Henry Ford

RECENT RESEARCH AWARDS:

Congratulations to:

- **Dr. Beaumont—Cyberonics—Acute and chronic evaluation of VNS for treatment of epilepsy.**
- **Dr. Champney—RDC—Dual antibiotic effects on bacterial ribosome synthesis.**
- **Dr. Ecay—RDC—Calcium nutrition in development 2:Mechanism of uptake by lizard embroys.**
- **Dr. Southerland—ITHACA--Remodeling of the guinea pig intrinsic cardiac plexus (no-cost extension).**
- **Dr. Thewke—NIH R15—Modulation of atherosclerosis by type 2 cannabinoid receptor.**

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RECENT JOURNAL PUBLICATIONS:

Chen P., Fan Y., Li Y., Sun Z.W., Bissette G. and Zhu M.-Y.: Chronic social defeat up-regulates expression of norepinephrine transporter in rat brains. *Neurochemistry International*, 2012, 60:9-20.

Daniels, C.R., Foster, C., Yakoob, S., Dalal, S., Joyner, W.L., Singh, M., Singh, K. Exogenous ubiquitin modulates chronic β-adrenergic receptor stimulated myocardial remodeling: role in Akt activity and matrix metalloproteinase expression. Am. J. Physiol. Heart and Circ. Physiol. 303:H1459-H1468, 2012.

Foster, C.R., Zha, Q., Daniel, L.L., Singh, M., Singh, K. Lack of ataxia telangiectasia mutated kinase induces structural and functional changes in the heart: Role in β-adrenergic receptor-stimulated apoptosis. Exp Physiol. 2012, 97:506-515. (*Article published with a viewpoint)* Ataxia telangiectasia mutated kinase in the heart: currency for myocyte apoptosis**.** Gorr, M.W., Stevens, S.C.W. and Wold, L.E. Exp Physiol. 2012, 97:476).

Frazier, A. and S. Champney, The vanadyl ribonucleoside complex inhibits ribosomal subunit assembly in *Staphylococcus aureus*. J. Antimicrobial Chemother. 2012 67(9): 2152-2157.

Frazier, A. and S. Champney. Impairment of ribosomal subunit synthesis in aminoglycoside treated ribonuclease mutants of *Escherichia coli*. Arch. Microbiology, 2012 194 (12): 1033-1041.

Fregoso, S.P. and Hoover, D.B**.** Development of Cardiac Parasympathetic Neurons, Glial Cells, and Regional Cholinergic Innervation of the Mouse Heart. Neuroscience 221: 28-36, 2012.

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Recent Journal Publications (Cont'd.)

Gang, T. B., D. J. Hammond Jr., S. K. Singh, D. A. Ferguson, Jr., V. K. Mishra, and A. Agrawal. The phosphocholine-binding pocket on C-reactive protein is necessary for initial protection of mice against pneumococcal infection. *J. Biol. Chem*. 287: 43116-43125, 2012.

Korossy-Mruk E, Kuter K, Nowak P, Szkilnik R, Rykaczewska-Czerwinska M, Kostrzewa RM, Brus R (Jan 2013) Neonatal DSP-4 treatment modifies antinociceptive effects of the CB1 receptor agonist methanandamide in adult rats. Neurotox Res 23(1), 39–48.

Ordway G.A., Szebeni A., Chandley M.J., Stockmeier C.A., Xiang L., Newton S.S., Turecki G., Duffourc M., Zhu M.-Y., Zhu H., and Szebeni K.: Reduced gene expression of bone morphogenetic protein 7 in brain astrocytes in major depression. Int. J. Neuropsychopharmacology, 15:855-868, 2012.

Phillips-Campbell, R., Kintner, J., Whittimore, J., and Schoborg, R.V. Chlamydia muridarum enters a viable but non-infectious state in amoxicillin-treated BALB/c mice. Microbes Infect. 2012 Nov;14(13):1177-85. PMID: 22943883.

Steagall, R.J., Sipe, A, Williams, C.A., Joyner, W., Singh, K. Substance P release in response to cardiac ischemia from rat thoracic spinal dorsal horn is mediated by TRPV1. Neuroscience 2012, 214:106-119.

Thirumalai, A., S. K. Singh, D. J. Hammond Jr., M. K. Pangburn, V. K. Mishra, D. A. Johnson, A. E. Rusiñol, and A. Agrawal. Exposing a hidden functional site of C-reactive protein by site-directed mutagenesis. *J. Biol. Chem.* 287: 3550-3558, 2012.

Thirumalai, A., B. Voleti, D. J. Hammond Jr., and A. Agrawal. Oct-1 acts as a transcriptional repressor on the C-reactive protein promoter. *Mol. Immunol.* 52: 242-248, 2012.

Wagner, R, S. Modla, F. Hossler, and K. Czymmek. "Three-Dimensional Analysis and Computer Modeling of the Capillary Endothelial Vesicular System with Electron Tomography" R. Wagner, Shannon Modla, Fred Hossler, and Kirk Czymmek. Microcirculation 19 (6), 477-484 (2012). (An image from the paper was used on the journal cover).

Zhang J., Fan Y., Li Y., Zhu H., Wang L., and Zhu M.-Y.: Chronic social defeat up-regulates expression of the serotonin transporter in rat dorsal raphe nucleus and projection regions in a glucocorticoid-dependent manner. *Journal of Neurochemistry*, 2012, 123(6):1054-1068.

Dean's Honorary M&M Research Award goes to:

Dr. Sharon Campbell, Principal Investigator (Mentors: Drs. Ardell and Ordway); *Determining the role of reactive oxygen species and vitamin E in depression.* $30,000; 1/1/13 to 12/31/13.

"I would like to express my sincere thanks to Drs. Jeff Ardell and Greg Ordway for the time and assistance they gave in the development of my project "Determining the role of ROS and vitamin E in depression." Their mentorship contributed greatly to the funding success. In addition, a special thanks to Dr. Russ Brown who has agreed to serve as a Co-PI to implement the psychological testing that will be required to carry out this research. I would also like to thank Dr. Phillip Musich and Dr. Koyamangalath Krishnan for their guidance that provided my background knowledge of this subject. Finally, I would like to express my thanks to Dr. Bagnell for his generosity in making the funding available to grow our research at Quillen." - Dr. Sharon Campbell.

Departmental (DBMS) M&M Awards go to:

Dr. Jennifer Hall, Principal Investigator (Mentors: Drs. Wyrick and Schborg); *Characterization of hormonal influences on Chlamydia trachomatis development.* $25,000; 1/1/13 to 12/31/13.

Dr. Russ Hayman, Principal Investigator (Mentor: Dr. Schoborg); *Encephalitozoon intestinalis infection increases host cell mutation frequency*. $25,000; 1/1/13 to o 12/31/13.

BOOK/CHAPTERS:

Chandley MJ and Ordway GA. Noradrenergic Dysfunction in Depression and Suicide. In: The Neurobiological Basis of Suicide. Chapter 3. (ed. Yogish Dwivedi) CRC press, Boca Raton. (2012)

Stone William L., V. P. Ramsauer, Campbell S.E., and Krishnan K. Targeted Prostate Cancer Chemoprevention Trial with Tocotrienols, p. 101-115. *In* Barrie Tan and V. R. P. Ronald Ross Watson (ed.), Tocotrienols: Vitamin E beyond Tocopherols. CRC Press Taylor and Francis, Boca Raton. (2013)

William L. Stone, Sharon E.Campbell, and Koyamangalath Krishnan. The Role of Vitamin E in Prostate Cancer, p. 333-354. *In* Donald R.Spitz, Kenneth J.Dornfeld, Koyamangalath Krishnan, and David Guis (ed.), Oxidative Stress in Cancer Biology and Therapy. Humana Press, New York. (2012)

INVITED MEETING PRESENTATIONS...

JENNIFER HALL (Assistant Professor) was an invited platform speaker at the Australian Chlamydia Conference in Brisbane, Australia. Title Citation: Hall, J. V., Kintner, J., Sun, J., Bambino, M., Novack, M., and Schoborg, R.V. "Involvement of Host Cell Signal Transduction Pathways in HSV-co-infection-induced *Chlamydia trachomatis* Persistence." Australian Chlamydia Conference. November 21, 2012. Brisbane, Australia.

ROBERT SCHOBORG (Professor) will be presenting one of the three keynote talks at the German Chlamydia Workshop in Würzburg in April 2013.

SCOTT CHAMPNEY (Professor). The Programme Committee of the 23rd European Congress of Clinical Microbiology and Infectious Diseases has announced that the abstract entitled, "Inhibition of protein synthesis and ribosome biogenesis in Staphylococus aureus, Haemophilus influenzae and Streptococcus pneumoniae by solithromycin, a new fluoroketolide," by W. Rodgers, A. Frazier, S. Champney, has been accepted for Publication; Berlin, Germany, Apr. 27-30, 2013.

Information to be included in the Newsletter should be submitted to T.J. Neal, Editor, by the 10th of each month.

S.O.S. Meeting

Next Session: Friday, February 22, 2013 at 12:00 p.m. – Small Auditorium, Stanton-Gerber Hall. Presenter: Dr. Antonio Rusinol.

Anyone interested in being a presenter, please contact Dr. Mike Kruppa, Seminar Committee Chair, to schedule a date.

IMPORTANT NOTICE

Next Quarterly DBMS Faculty Meeting

February 28th

3:30 p.m.

Large Auditorium

(Please put this date on your calendar)

RECYCLING...

Remember to donate your plastic bags to the "Bags to Benches" recycling program. If you want further information, please contact Bobbie Connelly@439-2053.

UPCOMING DBMS SEMINAR SCHEDULE:

Jan 21st Sean Fox - Student

Jan 31st (Rescheduled for Feb. 7th)

Feb 4th Regenia Philips – Student

Feb 7th Rakesh Kukreja (VCU)

Feb 25th Cory Leonard –Student

Mar 4th Chuan-Yuan Li (Duke)

Mar 26th Peter Sun (NIH

Apr 16th Todd Reynolds (UT-knox) -

Apr 22nd James Kaper (U. Maryland)- Student Invited Speaker

May 10th Dr. Blakely (Vanderbilt) - This is a joint engagement with Dept. of Psychiatry

November 30th—Dr. Matthew Shotwell—co-sponsored by Institute for Quantitative Biology.

Further details will be sent via email.

Dr. Mike Kruppa, Seminar Committee Chair.

Please contact Dr. Kruppa to schedule a seminar.

The *Biochemical Nature of Disease Journal Club* has been established. This club meets at noon on Thursdays and is a brown bag lunch. Topics are of speaker's choosing. The paper to be discussed is sent via email on Monday. Topics that have been discussed thus far related to antibiotic resistance, autophagy, translation control, DNA repair, lipid metabolism, apoptosis, mechanisms of cancer, heart disease and neuropathology. Any interested parties may contact Dr. Sharon Campbell if they would be interested in giving a presentation.

Below is a tentative schedule for Spring 2013:

January 10	Sharmi Mazumder
January 17	Ben Hilton
January 24	Jack Rary
January 31	Toh Gang
February 7	Natalie Burke
February 14	David Hurley
February 21	Avinash Thirumalai
February 28	Eliot Smith
March 7	Jaime Parman
March 14	Sharon Campbell
March 21	Laura Daniel
March 28	Hui Wang
April 4	Appalachian Student—Research Forum (No meeting)
April 11	Mitch Robinson
April 18	Annie (Yan Wang)
April 25	Maya Breitman
May 2	You Zou

Abstract/Project Summary for Recent Research Awards

ACUTE AND CHRONIC EVALUATION OF VNS FOR TREATMENT OF EPILEPSY

E. Beaumont, P.I.; J. Ardell, G. Ordway, and R. Brown, Co-P.I.

Chronic epileptic seizures dramatically modify the neuronal network integrity in the central nervous system and we hypothesize that VNS therapy will reverse these deleterious changes in patients over time. By using our unique expertise in recording from different neuronal population in the CNS and peripheral autonomic ganglia simultaneously, we will assess the rewiring of the neuronal circuitry in select CNS regions as a consequence of VNS. Such basic science data are necessary if one is to improve the understanding of the efficacy of VNS therapy and design new stimulus paradigms to maximize clinical efficacy. The ultimate goal is to move VNS up the treatment ladder while at the same time reducing morbidity and mortality associated with chronic epilepsy.

DUAL ANTIBIOTIC EFFECTS ON BACTERIAL RIBOSOME SYNTHESIS

S. Champney, P.I.

This is a proposal to investigate methods for reducing the continuing increase in the appearance of antibiotic-resistant microogranisms. We are using Staphylococcus aureus cells to study the effect of dual antibiotic treatment on the synthesis of ribosomes in these cells. Azithromycin, a protein synthesis inhibitor is used in combination with ciprofloxicin, a DNA synthesis inhibitor or with rifampicin, an RNA synthesis inhibitor. A triple drug procedure is also being used. Antibiotic inhibition of multiple cellular targets should significantly reduce the appearance of resistant organisms.

CALCIUM NUTRITION IN DEVELOPMENT 2: MECHANISM OF UPTAKE BY LIZARD EMBRYOS

T. Ecay, P.I.

Calcium is an essential dietary component at all stages of life. Our research seeks to understand how vertebrate embryos obtain calcium needed for development. The European Common Lizard is unique as it has egg-laying and live-bearing populations. We have shown that embryos of live-bearing females receive most of their calcium from placental transport and the embryonic mechanism for calcium transport involves specific proteins that bind calcium to aid the transport process. How expression of specific proteins responds to variation in calcium availability is unknown. This process is difficult to study as embryos develop within the female. We have developed a protocol to remove embryos from females and incubate them under conditions that we can experimentally manipulate. We will use this protocol to determine at what age embryos begin to acquire calcium and how they regulate transport. The project is organized into two phases. In phase I, we will expose embryos to solutions with different calcium and quantify the timing and mass of calcium uptake. This will reveal the developmental age at which embryos take up calcium. In phase II, we will analyze tissue expression of calcium transport proteins from the same embryos to assess their response to varying calcium availability .Our results will be used to formulate an hypothesis to explain the role embryos play in maintaining healthy calcium nutrition. Female lizards will be available in 2013 but may be limited in subsequent years.

Abstract/Project Summary for Recent Research Awards

REMODELING OF THE GUINEA PIG INTRINSIC CARDIAC PLEXUS

E. M. Southerland, P.I; J. Ardell, Co-P.I.

Previous studies have shown that an imbalance in autonomic efferent neuronal tone, with reduced parasympathetic activity coupled with increased and heterogeneous sympathetic outflow, increases the risk of cardiac arrhythmias and sudden death. While the progression of cardiac disease affects multiple aspects of cardiac control, it is those changes in peripheral autonomic neuronal processing and their projections that ultimately determine the neuronal coordination of the heart. The intrinsic cardiac nervous system (ICN), the final common pathway for such neural control, integrates information from multiple inputs and mediates short-loop reflex control of regional cardiac indices. Although multiple studies have focused on cardiac stress-induced changes in post-ganglionic innervation patterns to the heart, little attention has been paid to the critical role of information processing within autonomic ganglia and how they remodel/adapt to imposed stress. It is our hypothesis that cardiac stress-induced adaptations within the ICN facilitate coordination of efferent parasympathetic output and that these changes are reflected in functional and phenotypic alterations in select intrinsic cardiac neuronal populations. These cardioprotective adaptations within the ICN could counteract, in part, the maladaptive effects of excessive sympatho-excitation associated cardiac stress.

Numerous studies have identified molecular mechanisms associated with cardiac remodeling, including increased activity of the renin-angiotensin system, changes in nitric oxide (NO) production, and alterations in end-organ sensitivity to neurotransmitters. For each of these factors, while their direct effects on myocyte function are well established, recent data indicates that many of their cardiac effects are mediated via alterations in function within the cardiac nervous system. To specifically address these points this proposal will evaluate how the elements of the ICN adapt to chronic disease using two different animal models of heart disease: myocardial infarction (MI) and chronic pressure overload (PO). The proposed experiments will focus on two specific adaptations of the ICN: (1) changes in neuronal responses to neuromodulators and (2) changes in ICN network efficiency. We will also evaluate the efficacy of targeted pharmacologic therapy to mitigate adverse remodeling of the ICN. Using a whole mount preparation of the guinea pig cardiac plexus, we will evaluate the physiological responses of individual intrinsic cardiac neurons to autonomic neurotransmitters with and without potential neuromodulators, such as angiotensin II and NO in tissues from control, MI and PO animals to characterize stress-induced changes in neuronal responses. In addition, we will evaluate the output of individual neurons to stimulation of vagal and intraganglionic fiber inputs to evaluate integrated network function. Changes in neuronal activity will then be compared between untreated disease models and disease models treated with standard therapeutics such as β-receptor blockage, AT receptor inhibition, or inhibition of NO generation, to determine if these therapies modulate the ICN function.

Abstract/Project Summary for Recent Research Awards

MODULATION OF ATHEROSCLEROSIS BY TYPE 2 CANNABINOID RECEPTOR

D. Thewke, P.I.; A. Rusinol and S. Brown, Co-P.I.

Atherosclerosis is characterized by the build up of fat, cholesterol, and immune cells in the walls of arteries to form plaques. When the surface of a plaque weakens, it can become unstable and rupture producing a heart attack or stroke, the leading cause of death in the US. The long-term goal of this research is to identify cellular mechanisms modulating atherosclerotic plaque in order to find new ways of treating atherosclerosis. Cannabinoids are compounds related to the active ingredient in marijuana and exert their effects by binding to cell surface receptors called cannabinoid receptors. Recent studies have shown cannabinoid receptors are dysregulated in a number of different inflammatory conditions, including atherosclerosis. Mice lacking the type 2 cannabinoid receptor, known as CB2, develop atherosclerotic plaques with evidence of reduced stability. However, the precise functions of CB2 in different stages of atherosclerosis are unclear, and it is unknown if targeting CB2 will trigger disease progression or provide a protective effect on plaque stability. The overall objectives of this research are to define the functions of CB2 in atherosclerosis and determine the effects of selectively targeting CB2 on plaques in atherosclerosis-prone mice. The central hypothesis is that macrophage CB2-dependent processes affect plaque stability, and that administration of CB2-selective compounds will provide beneficial effects on atherosclerosis. To test this hypothesis we propose the following specific aims: 1) Identify CB2-dependent effects on lesion stability in atherosclerosis-prone mice, 2) Identify CB2-dependent effects on atherosclerotic lesion calcification mechanisms in mice, and 3) Identify the cell types contributing to CB2-dependent effects on atherosclerosis in mice. Each aim will be tested using atherosclerosis-prone mice which lack CB2 and by treating atherosclerosis-prone mice with compounds to selectively activate or inhibit CB2. This research is innovative because no data currently exist regarding the effects of CB2 in advanced atherosclerosis or how CB2-selective compounds will affect atherosclerosis. Ultimately, the knowledge gained from this project will advance the field of atherosclerosis research by providing information necessary to determine if CB2 is a viable target for the design of potentially new therapies to treat atherosclerosis in humans.

Abstract/Project Summary for Recent Research Awards

DETERMINING THE ROLE OF REACTIVE OXYGEN SPECIES AND VITAMIN E IN DEPRESSION

S. Campbell,, P.I., R. Brown, Co-P.I. (Mentors: J. Ardell and G. Ordway)

Despite 50 years of treatment with anti-depressants that restore noradrenergic and serotoninergic neurotransmitter systems, there still is a gap in understanding what biochemical mechanisms initiate major depressive disorder (MDD). The continued existence of this gap is an important problem because until the biochemical mechanism behind depression is realized, the development of drugs that can target these biochemical factors is hindered. As MDD disrupts more than 120 million lives globally and places a burden on the U.S. economy of more than $80 billion per year, it is imperative to understand the biochemical process leading to depression. The objective in this application is to determine which reactive oxygen (ROS) and nitrogen species (RNS) are liberated during a chronic stress paradigm in an animal model predictive of depression, and to determine whether common antioxidant (vitamin E) can simultaneously block the ROS/RNS and behavior deficits associated with the chronic stress paradigm. This will be accomplished using the social defeat model in C57BL/6 mice. This model is an excellent model to study the development of depression as it is well-established that mice exposed to this level of social stress, develop "depressive-like" symptoms that become apparent when challenged with the behavioral assays such as the force swim test and/or the tail suspension test. The central hypothesis is the production of ROS and/or RNS play a central role in the development of depression that vitamin E isoforms can prevent or slow the pathological process leading to depression by free radical scavenging. This hypothesis has been formulated based on literature showing increased down-stream biochemical markers of oxidative stress in the tissues of MDD patients compared to control subjects. These biomarkers include increased lipid peroxidation and increased expression of TNF-alpha, interleukin 8, and interleukin 6. In addition, MDD patients have polymorphisms in oxidative stress enzymes and these polymorphisms are associated with increased risk for depression. Serum alpha tocopherol concentrations are lower in patients with MDD suggesting a role for tocopherols in the protecting the brain from the development of depression. Further, alpha tocopherol produces an "anti-depressant-like effect" in predictive animal models of acute depression. The rationale for the proposed research is that understanding the biochemical process leading to MDD will allow for the development of agents that can target the initiation of MDD which can be used alone or in combination with current therapies to reduce the prevalence and persistence of MDD. Guided by strong literature data this hypothesis will be tested by using the following specific aims: 1) to determine the time frame of the liberation of the ROS and RNS during the delivery of social defeat stress 2) to determine if vitamin E (alpha or gamma tocopherol) reduce the depressive-like symptoms associated with the social defeat stress by reducing formation of the ROS and RNS. C57BL/6 mice will receive diets that are deficient in Vitamin E or have been supplemented with alpha or gamma tocopherol prior to and throughout the social defeat stress. CNS microdialysis probes will be implanted into the brain of each mouse to attain extracellular fluid for the measure of ROS and RNS. The extracellular fluid will be collected at varying times following the stress (0, 0.5,1, 2, 5,10, and 12days) and analyzed for ROS and RNS. At days 2 and 10 of the social defeat stress the animals will be subjected to behavioral assays to measure the degree of "depressive-like" response from each dietary group. The average biochemical responses ($O_2\cdot^-$, $HO\cdot^-$, H_2O_2 and NO concentrations) and immobility responses (measure of anhedonia from the force-swim test) for each dietary group at each time will be compared using an ANOVA followed by a Tukey's multiple comparison test ($p < 0.05$ is considered significant). This approach is innovative because it utilizes microdialysis probes to attain multiple measures of oxidative stress in each animal through the development of "depressive-like" symptoms rather than the studying the end result of depression. The proposed research is significant because it will vertically advance the understanding of the types of ROS and RNS produced during different stages of the development of depression. It will provide quantitative data of ROS and RNS that can be correlated with the degree of depression. Further it will demonstrate whether dietary Vitamin E isoforms have anti-depressant properties in the presence of a chronic stress model of depression. The knowledge gained from this study can be used to target specific ROS and RNS involved in the development of depression and may ultimately lead to new treatments that reduce the burden of MDD on society.

Abstract/Project Summary for Recent Research Awards

CHARACTERIZATION OF HORMONAL INFLUENCES ON *CHLAMYDIA TRACHOMATIS* DEVELOPMENT

J. V. Hall, P.I. (Mentors: P. Wyrick and R. Schoborg)

Increased levels of the hormones, estrogen and progesterone, increase occurrence and/or severity of sexually transmitted infections. In the US, *C. trachomatis* annually causes 2,800,000 new infections and remains the most frequent bacterial sexually transmitted infection worldwide. The non-invasive, sexually transmitted *C. trachomatis* serovars D-K are obligate intracellular pathogens that replicate primarily within genital tract (GT) luminal and glandular epithelial cells. If untreated, these organisms can ascend the GT, evoking pelvic inflammatory disease, infertility and ectopic pregnancy in infected women. Clinical trials, animal models and *in vitro* studies demonstrate that estrogen enhances chlamydial infection. Steroid hormones have both direct and indirect effects on human endometrial epithelial cells. Estrogen and progesterone exposure induces endometrial gland stromal cells to release effector molecules that subsequently regulate growth and maturation of uterine epithelial cells. Dr. Hall has investigated the effects of estrogen on chlamydial infection in an endometrial epithelial cell (Ishikawa or HEC-1B)/stromal cell (SHT-290) co-culture system, which provides the unique opportunity to dissect complex hormone-stromal cell-epithelial cell interaction in a simplified *in vitro* setting. Previous data indicate that: i) membrane-associated estrogen receptors (mERs) help mediate *C. trachomatis* serovar E entry into host genital epithelial cells; ii) estrogen receptor signaling facilitates intracellular development of *C. trachomatis*, and; iii) estrogen-stimulated endometrial stromal cells secrete effectors that indirectly aid intracellular chlamydial development in genital epithelial cells. The current proposal will investigate the effects of both estrogen and progesterone on chlamydial infection. During the menstrual cycle, estrogen concentrations peak during the proliferative phase, while progesterone concentrations peak during the secretory phase. Both hormones are present at varying ratios at other times in the cycle. Aim 1 will test the hypothesis that progesterone antagonizes the effects of estrogen upon chlamydial development. Aim 2 will test the hypothesis that the secreted stromal cell effector, osteopontin, influences *C. trachomatis* development in co-cultured epithelial cells. Data from these experiments will: i) produce a solid foundation for NIH R15 or R21 proposals; ii) provide further characterization of a physiologically-relevant *in vitro* infection model of the female endometrium; and iii) increase understanding of how hormones modulate chlamydial infection, transmission and reproductive pathology.

ENCEPHALITOZOON INTESTINALIS INFECTION INCREASES HOST CELL MUTATION FREQUENCY

R. Hayman, P.I. (Mentor: R. Schoborg)

Microsporidia are obligate intracellular opportunistic fungi that can cause significant pathology in immunocompromised patients. Transmitted by contaminated water, the organisms initially infect the gastrointestinal enterocytes ultimately leading to diarrheal disease. Some species of microsporidia, such as *Encephalitozoon intestinalis*, can disseminate from the intestines to reside in almost any organ in the body. It is now recognized that immune competent people are also exposed to microsporidia as 11% of the general population have antibodies to one or more species. The mammalian cells that become infected with microsporidia undergo severe stresses. The cells display increases in ribosomes, endoplasmic reticulum, and mitochondria. In some cases, the cells become multinucleated. It has also been shown that infected individuals have elevated levels of oxidative stress markers, such as hydrogen peroxide and free radicals. These toxic components have been strongly linked to cancer and aging. The presence of these stressors lead the mentee to speculate that host cell nuclear mutation rates may increase in infected cells and/or in surrounding uninfected cells. It is, therefore, **hypothesized that 1) microsporidia infection *in vitro* will increase the DNA mutation frequency with host cells; and 2) microsporidia infection of host cells will alter the expression and localization of select host DNA repair and DNA damage checkpoint proteins**. In the first Aim of this proposal, mutation frequencies will be determined using microsporidia infected fibroblasts from "BigBlue" mice, which contain DNA target sites that allow phage to differentially plaque at 24^{o}C if the target becomes mutated. From these experiments, mutation frequencies can be determined in infected versus mock-infected cells. In the second Aim, the expression and localization patterns of select DNA repair and DNA checkpoint proteins will be determined by Western blotting and immunofluorescence assays. Altered repair and checkpoint proteins are additional indicators of nuclear DNA damage. The concept that microsporidia infection may cause DNA damage to host cells is novel. And, although the data linking microsporidia to cancer is limited, the data linking immunosuppression and other intracellular pathogens to cancer is convincing. This proposal will open a new avenue of research for the mentee, which will lead to future NIH proposals.