

**Leadership Program for Veterinary Students  
at Cornell University**

**1996 ANNUAL REPORT**



The College of Veterinary Medicine's 1996 Leadership Program for Veterinary Students was designed to provide veterinary students with the opportunity to develop leadership skills and to gain experience in an academic setting. The program was designed to provide students with the opportunity to develop leadership skills and to gain experience in an academic setting.

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## **Report of the 1996 Leadership Program**

### **for Veterinary Students**

#### **College of Veterinary Medicine**

#### **Cornell University**

Participants in the Leadership Program for 1996 included: Boston (one), Frankfurt (one), Ames (one), Margaret Yllescu, Justin Smay, Nyma Marwan, Miriella Davis, Jessica Green and Emily Peterson. Second year: Patricia DeGroot, Felicity Cole, Lisa van Nieuwen, Vikram Narayan, Susan Blum, Sarah Marshall, Jennifer West, Mylene Morgan (coordinator), Thomas Gell and Linda Rhodes (coordinator). Top row: Wendy McGregor (program director), Peter Ryan (coordinator), Linda Griebel (graduate education coordinator), John Van, James Fletcher (coordinator), Benjie Mangoni, Mark O'Brien, Jonathan Green, Ralph S. Green, Roger Antonio Jettison, Constantine Van der Bruggen, Alice Lerner, Edith van der Grinten and Allen Kasper (coordinator).

The program spanned six weeks, during the months of June, July and August. It featured faculty guided research and group learning experiences in which the students assumed major responsibility for organizing modules and running an ongoing group of discussion leaders and coordinators.

Students were assigned individual research projects that enabled them to explore a variety of subjects, to learn new investigative strategies, and to gain insight into how a research laboratory utilizes its professional and material resources. The students also had a variety of individual and group learning assignments. These included the development of leadership, communication and critical thinking skills, as well as the ability to work cooperatively with the proper support of research. Site visits to the national institutes of Health and





## Program Overview

The College of Veterinary Medicine at Cornell University hosted the seventh annual Leadership Program for Veterinary Students this year. The program targets students who have the potential to be veterinary decision makers and leaders of their profession. It is anticipated that many will realize their careers in an academic setting or as research scientists in government or industry.

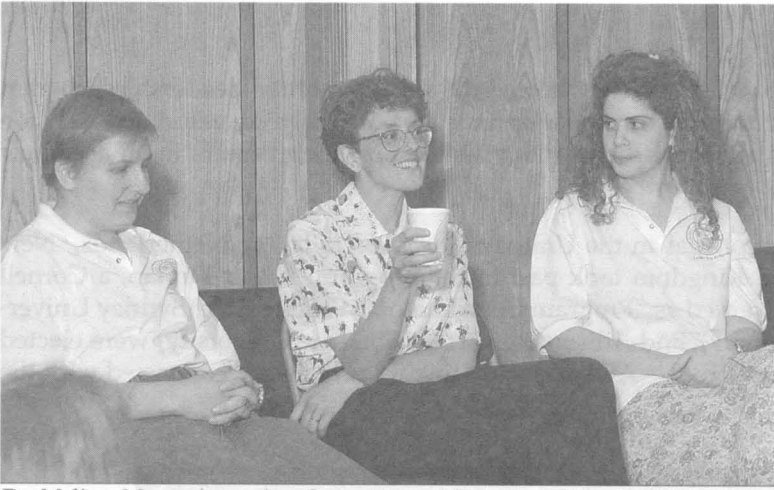
Twenty-four students from 18 veterinary colleges in the United States, Australia, the Netherlands, New Zealand, South Africa, Spain, and the United Kingdom took part in the program. Allan Kaplan, a Cornell undergraduate and aspiring medical student, served as "Program Coordinator." Felicity Cole (Sydney University), Patricia Gearhart (Michigan State University), and Allison Stewart (Melbourne University) were elected by their fellow students as "Program Representatives." All had facilitating roles in events connected with the program.



**Participants in the Leadership Program for 1996 included: Bottom row, from left: Amy Schein, Margaret Fleischli, Justine Swaney, Nyree Beeston, Michelle Dries, Jessica Geyer and Polly Peterson. Second row: Patricia Gearhart, Felicity Cole, Ilse van Vonderen, Allison Stewart, Sarina Hinsley, Suzanne McNabb, Jennifer Hess, Melissa Mazan (counselor), Tamara Gull and Linda Rhodes (counselor). Top row: Douglas McGregor (program director), Peter Eyre (counselor), Linda Griswold (graduate education coordinator), John Stein, James Flanders (counselor), Bernice Mangnall, Mark Doherty, Jonathan Goodwin, Ralph Senften-Rupp, Antonia Jameson, Constantin Von der Heyden, Alicia Uixera, Edwin van Duijnhoven and Allan Kaplan (program coordinator).**

The program spanned ten weeks during the months of June, July and August. It featured faculty guided research and group learning experiences in which the students assumed major responsibility for organizing modules and hosting an outstanding group of discussion leaders, and consultants.

Fellows were assigned individual research projects that enabled them to explore a variety of subjects, to learn new investigative strategies, and to gain insight into how a research laboratory utilizes its professional and material resources. The students also had a variety of individual and group learning assignments. These focused on creativity, the development of leadership, communication and critical thinking skills, as well as ethical issues connected with the proper conduct of research. Site visits to the National Institutes of Health and



*Dr. Melissa Mazan (center) at Career Day with students Tamara Gull (left) and Jennifer Hess (right).*

the United States Department of Agriculture were additional features of the program.

Career counseling occurred frequently and informally while the program was in session, but an entire day was devoted to this activity in a more structured setting. Four veterinarians who have achieved distinction as research scientists and administrators, or who are advanced in their own training, served as discussion leaders. Taking part this year were:

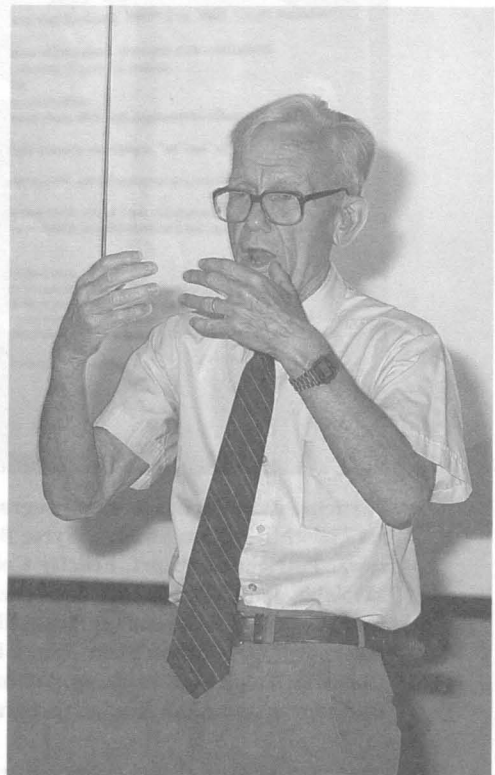
- Dr. Peter Eyre, Dean of the Virginia-Maryland Regional College of Veterinary Medicine
- Dr. James A. Flanders, Ph.D. Graduate Student, Department of Pharmacology, College of Veterinary Medicine, Cornell University
- Dr. Linda Rhodes, Senior Research Fellow, Department of Pharmacology, Merck and Company
- Dr. Melissa Mazan, Resident in Large Animal Medicine, College of Veterinary Medicine, Tufts University.

Professors Barry J. Cooper and N. Sydney Moise led an evening discussion of internship and residency training. The discussion identified factors that veterinary students should weigh in evaluating residency programs in pathology and the clinical sciences. On another occasion, Professors Richard A. Cerione, Noa Noy, Edward J. Pearce, and Karel A. Schat addressed similar issues connected with graduate research training. At still another meeting, two scientists from the research laboratories of Merck & Company, Dr. Gerard J. Hickey and Dr. Michelle L. Haven, discussed career opportunities for veterinarians in industry. They also commented on their research and administrative duties at Merck and Co., highlighting the similarities and differences between research conducted in an academic institution as opposed to a research-intensive pharmaceutical company.

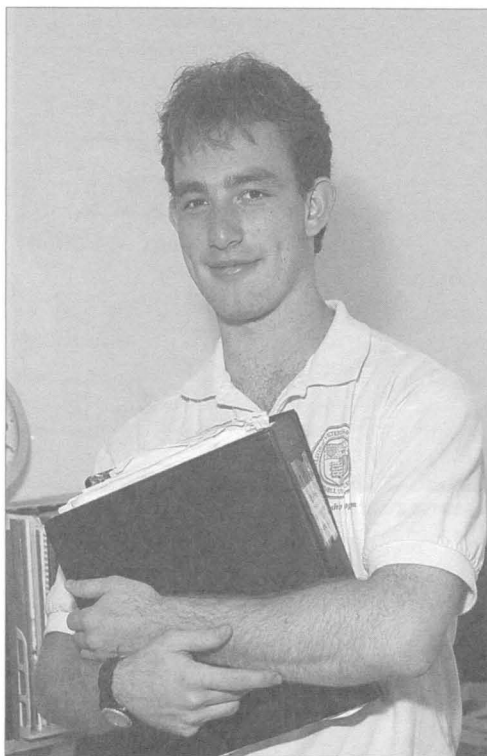
Dr. Ari van Tienhoven, Emeritus Professor of Animal Physiology, has had a prominent role in the Leadership Program for several years. The project he organized this year focused on the scientific method. It provided students practical experience in reviewing scientific manuscripts while simultaneously addressing ethical issues connected with the editorial process.

Dr. Marian Horzinek, Professor of Virology at the University of Utrecht, organized a new module that underscored the importance of "lateral thinking" as a characteristic of creativity in both science and the visual arts.

Many events connected with the program enable students to reflect on the characteristics and responsibilities of leadership. These issues were considered this year in a panel discussion moderated by Dr. Franklin M. Loew, Dean of the College of Veterinary Medicine at Cornell. The panel members were Dr. Judith L. Vaitukaitis, Director of the National Center for Research Resources at the National Institutes of Health (NIH) and Dr. Purnell Choppin, President of the Howard Hughes Medical Institute. The panel members responded to student com-



*Professor Ari van Tienhoven lecturing on "Ethics and the Scientific Method"*



Allan Kaplan, a Cornell undergraduate, served as Program Coordinator.

ments and questions about leadership, often in the context of their own experience. The discussion reinforced aspects of career planning which the students had considered in other modules.

Radioisotopes, hazardous chemicals and infectious agents are used frequently in veterinary research; yet many inexperienced investigators are unfamiliar with procedures connected with their proper acquisition, use and disposal. Participants in the 1996 program received four hours of instruction in radiation safety. The presentations were arranged by Cornell's Radiation Safety Officer, Mr. Thomas J. McGiff.

Dr. Larry J. Thompson, Director of Biosafety in the Veterinary College, presided at a meeting that focused on principles governing the handling, storage and disposal of hazardous chemicals. He also reviewed College policy regarding the protection of employees from rabies virus and other infectious agents.

Site visits to other institutions that are known for the excellence of their research and research training are a feature of the Leadership Program. This year the students and several faculty members at Cornell visited the NIH and the United States Department of Agriculture's (USDA) Livestock and Poultry Research Institute, both in the Washington, DC area. Agendae for the visits are reproduced below.

**National Institutes of Health  
June 13, 1996  
Research and Research Training at the NIH**

Ms. Lori Mulligan, NCRR Public Affairs Specialist, "Welcome, NCRR Video"  
 Ms. Pamela Dressell, Chief, Client Relations, VRP, "Overview of NIH, NCRR and NIH Veterinarians"  
 Dr. C. Max Lang, Director, VRP, "Overview and Interrelationship of NIH, NCRR, and VRP"  
 Dr. Victoria Hampshire, Chief, Carnivore and Ungulate Section, Research Animal Branch, VRP, "Gene Therapy — Local and Systemic Approaches"  
 Dr. Georgia Miller, Veterinary Pathologist, Pathology Unit, Laboratory Sciences, VRP, "Hepatic Hemosiderosis in Marmosets"  
 Dr. Tony Wynchaw-Borris, Director, Mouse Models Section, NCHGR, "Tour of Transgenic and Knockout Mice Laboratory"  
 Dr. Clarence Gibbs, Deputy Chief, Laboratory of Central Nervous System Studies, "Transmissible Dementias in Humans and Animals"  
 LUNCH  
 Dr. Robert Donahue, Director, Simian Gene Transfer Program, "Primate Gene Transfer and Bone Marrow Transplantation Program" Tour of Facilities  
 Final Review

**United States Department of Agriculture  
(Beltsville Campus)  
June 14, 1996**

Dr. H. Ray Gamble, Acting Institute Director, "Overview of Beltsville and the Livestock and Poultry Sciences Institute"  
 Dr. Larry Johnson, Germplasm and Gamete Physiology Lab, "Sex Preselection in Livestock"  
 Dr. Caird Rexroad, Gene Mapping and Evaluation Lab, "Transgenic Livestock Production"  
 Break  
 Dr. Joan Lunney, Immunology and Disease Resistance Lab, "Genetics of Resistance to Livestock Parasites"  
 Dr. Ray Gamble, Parasite Biology and Epidemiology Lab, "Preharvest Control of Zoonotic Parasites"

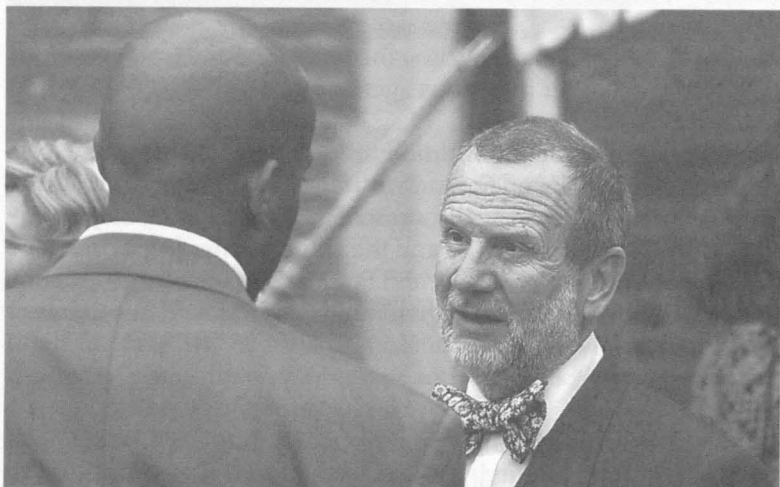
At the conclusion of the program, the students reported on their research activities. The presentations were uniformly good, and some were outstanding. Four students were awarded book prizes:



- Polly Peterson - Program Prize
- Margaret Fleischli - Molecular Biology Prize
- Tamara Gull - Cell Biology Prize
- Justine Swaney - Veterinary Medicine Prize

University of Sydney students presented their reports as a videoconference to an audience in Sydney, Australia. A second videoconference afforded an opportunity for students enrolled in an affiliated program at Sydney University to describe their research findings to their counterparts at Cornell.

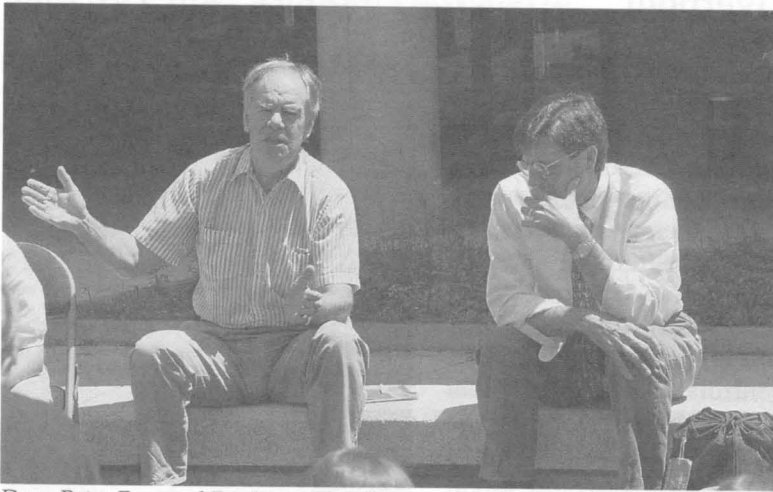
In the following narrative, the student fellows introduce themselves and describe their individual and collective experiences.



*Professor Marian Horzinek (right) speaking with Leadership Program student, Jonathan Goodwin (left).*



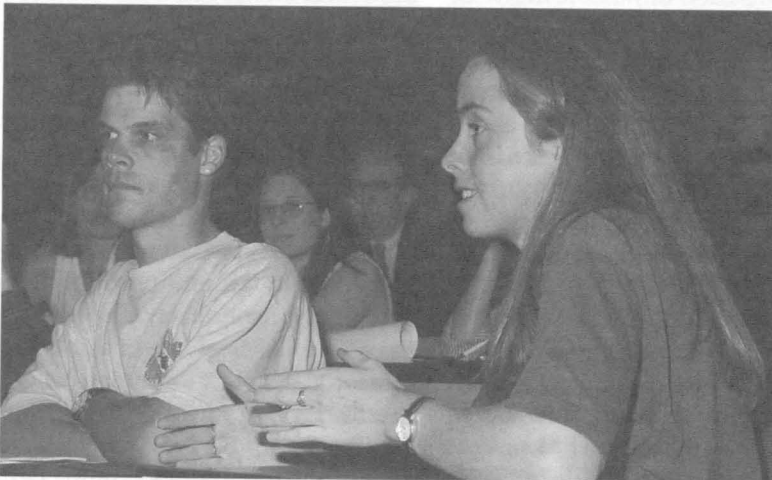
*Mark Doherty, Patricia Gearhart, Bernice Mangnall and Suzanne McNabb at the Career Day meeting.*



Dean Peter Eyre and Dr. James Flanders served as "Career Day" counselors.



Dr. Judith Vaitukaitis, Dean Franklin Loew, and Dr. Purnell Choppin led the students in a discussion of "Leadership."



Ralph Senften-Rupp and Felicity Cole directed questions to the panelists during the Leadership discussion."



## Nyree Beeston - University of Liverpool



As a veterinary student about to enter my final year of study at the University of Liverpool, England I began thinking about my career plans for the future. Although interested in clinical practice, I was anxious to discover what other options would be available to me as a qualified veterinarian, and what steps I should take in order to pursue these avenues. My introduction to scientific research came when I took a year out of my veterinary course to study the honors year of BSc. genetics, the practical element of which investigated the effects of antisense ribosomal RNA on mosquito oogenesis.

During my time at Cornell I have been working in Dr. Nixon's Comparative Orthopaedics laboratory. The research being carried out here focuses on developing growth factor and surgical grafting methods to enhance cartilage repair and prevent arthritis development, especially in horses.

The balance between the chondrocyte synthetic modulators, insulin like growth factor-1 (IGF-1) and transforming growth factor- $\beta$  (TGF- $\beta$ ), and deleterious agents such as interleukin-1 (IL-1) and metalloproteases, maintain the con-

dition of articular cartilage. Previous work by this laboratory has determined that minute quantities of IGF-1 and TGF- $\beta$  have a stimulatory effect on chondrocytes and may prove to be a therapy to counteract the deleterious effects of elevated levels of IL-1 and degradatory enzymes in osteoarthritis. However, as yet little is known about the regulatory control of IGF-1 and TGF- $\beta$ .

My project was to study the autoregulation of IGF-1 and cartilage matrix gene expression by exogenous IGF-1 application. Cartilage explants collected from the elbow and shoulder joints of a euthanased horse were cultured in media with IGF-1 at two different concentrations. After five days, the cultures were stopped and the products were analyzed for RNA, DNA, collagen and glycosaminoglycan (GAG). A quantitative evaluation of the RNA produced was obtained by isolating total RNA from cartilage tissue. The latter was then analyzed by quantitative PCR. A distributional analysis of IGF RNA production was carried out using *in situ* hybridization with IGF-1 and collagen type II antisense probes, with sense probes and RNase treated tissue used as controls. The GAG content of the cartilage was measured by absorbance of visible light at spectrophotometry following the addition of DMMB dye, and the microscopic location of GAG expression was established by staining with H&E and with Alcian blue and counter staining with nuclear fast red. Fluorescence spectrophotometry following the addition of Hoechst 33258 was used to ascertain the DNA content. Finally the collagen content was measured by HPLC.

My data showed that exogenous IGF-1 application has a negative feedback effect on IGF-1 mRNA expression in equine cartilage explants resulting in down-

regulation of IGF-1 transcription in a dose dependant manner. They also supported previous studies demonstrating that IGF-1 application, results in increased proteoglycan expression especially in the chondrocyte pericellular area.

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This summer allowed me to learn many new molecular biology techniques, to work as part of an enthusiastic and helpful team using equipment to which I would otherwise never have had access. I have also received advice regarding career choices that will be available to me upon finishing my bachelor of veterinary science and I have learned much about the veterinary science and cultures of other countries.

I would like to thank Dr. Nixon and his team for all of their help and encouragement, especially Dr. Lisa Fortier for all her guidance. I would also wish to express my gratitude to all the other people who have made this summer possible, the Wellcome Trust for their sponsorship, Dr. McGregor and Ms. Linda Griswold for all of their help, and last but by no means least, all the other leadership students who have helped make this summer so enjoyable.

## Felicity Cole - Sydney University



Great excitement and anticipation accompanied me when I came to Cornell University. As a fourth year at Sydney University I was keen to learn about opportunities in veterinary research. Working in Dr. Robertshaw's physiology laboratory provided an array of new experiences. I had never imagined a Poll Dorset ewe running at great speed on a treadmill; but as our work unfolded this unusual concept became a familiar sight, along with many other aspects of research.

Sheep are panting animals. In response to increasing body temperature they increase deadspace ventilation to dissipate heat and at rest they maintain alveolar ventilation. When sheep exercise two demands are placed on their respiratory system. First, they need more oxygen to fuel exercise. Second, they must dissipate heat produced during exercise. Under these conditions alveolar ventilation increases causing a hypocapnoea that is proportional to the heat load. The induced respiratory alkalosis results in a left shift in the oxygen dissociation curve for haemoglobin (Bohr effect) and haemoglobin affinity for oxygen increases. However, at increased body or muscle tempera-

ture haemoglobin has lower affinity for oxygen. My project was to determine if either the alkalosis or hyperthermia affect transfer of oxygen by exercising muscles.

Two sheep were studied on three occasions. Each experiment involved a sheep running on the treadmill for 30 minutes at constant speed. Blood was sampled at several intervals before, during and after exercise. Carotid arterial and external iliac venous blood were taken so changes across the leg could be measured. A blood gas analyzer was used to obtain  $pO_2$ ,  $pCO_2$ , and pH values. Oxygen content of blood was directly measured with a special  $O_2$  analyzer. Blood lactate and packed cell volume (PCV) values were also obtained. An ultrasonic flow probe around the external iliac artery measured blood flow so that oxygen delivery to the hind leg could be calculated. Rectal temperature and total oxygen consumption ( $VO_2$ ) of the animal were recorded. The six experiments produced repeatable results.

Several factors were found to affect the oxygen delivery to exercising muscle. An increase in blood flow to the leg from a resting value of 0.5 to 2.5 litres per minute during exercise was observed. PCV also increased from the resting value of 20% to 30% during exercise, hence increasing the oxygen carrying capacity of blood. These factors combined with an elevated  $paO_2$  increase the delivery of oxygen to the leg during exercise independent of pH or temperature. Other factors affected the uptake of oxygen at the cellular level. Oxygen content divided by PCV gave the amount of  $O_2$  per unit of haemoglobin. Arterial and venous values were compared with time,  $pCO_2$ , temperature and  $pO_2$ .

During exercise, the venous  $O_2$  per unit haemoglobin remained constant despite a rising pH and

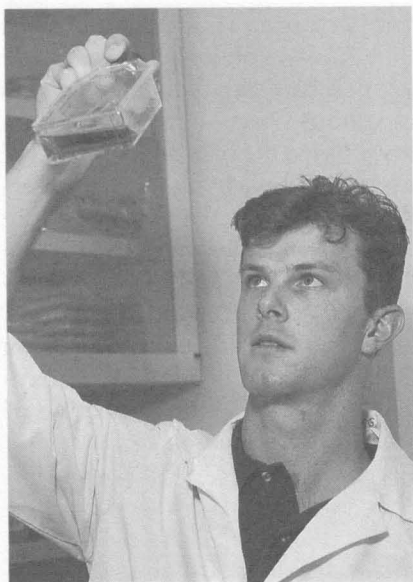
temperature. This demonstrated that the  $O_2$  uptake is unaffected by temperature or alkalosis. Changes in venous  $O_2$  content were only in response to the onset or cessation of exercise. The driving force transferring oxygen to the muscle is the  $pO_2$ . As muscles work, they metabolize oxygen and local  $pO_2$  declines. It was concluded that haemoglobin offloads oxygen in areas of low  $pO_2$  and hence delivers oxygen to the muscle irrespective of local pH or temperature.

Consistent with this conclusion is the fact that lactic acid production by the exercising limb did not increase. Throughout the course of experiments, however, arterial lactate levels rose suggesting that non exercising tissues are relatively hypoperfused in proportion to their oxygen consumption. As hyperthermia develops their oxygen requirements increase, and this is not matched by an increase in blood flow to these tissues.

The assistance and enthusiasm of my mentor, D. Robertshaw, made such a difference to my experience, that I am extremely grateful to him. Dr. Rawson was a valued ally as I tackled technicalities, and his kindness and support were appreciated.

Living with 23 other leadership students was truly fantastic. I am pleased to have had the chance to get to know such a marvellous group of people and make some great friends. All things would not have been possible without Dr. McGregor, Linda and Allan, and they have my sincerest thanks. I am grateful to the Gould Foundation for my sponsorship. Finally, I thank Professor David Fraser, our Dean in Sydney, for his friendship and endless support and for introducing me to this experience of a lifetime.

## Mark Doherty - Sydney University



The diversity of opportunities for Veterinary graduates, together with a desire to work with animals, attracted me to a career in veterinary medicine. The Cornell Leadership Program was appealing to me as an opportunity to explore the research side of the field. So with financial support of the USDA, I packed my bags, and headed for Dr. Edward Pearce's lab in the Department of Microbiology and Immunology at Cornell.

The lab works with the parasite *Schistosoma mansoni*, which is responsible for the debilitating disease in humans, schistosomiasis. Adult parasites live in the mesenteric veins where the females lay eggs. The eggs penetrate the intestinal wall and are expelled in the feces. New hosts become infected when the freshwater stage of the parasite penetrates the skin. Disease is caused by a granulomatous response to eggs which become trapped in the liver.

After the discovery of a surface receptor on *S. mansoni* related to the TGF- $\beta_1$  receptor, it was postulated that the parasite may also produce a TGF- $\beta$ -like protein. My project involved searching for a protein in the parasite with TGF- $\beta$  activity. To

do this I used an indicator cell line (mink lung epithelial cells), the proliferation of which is markedly inhibited by TGF- $\beta$ .

In the first few weeks of the program I developed a protocol to measure the effect of TGF- $\beta$  on epithelial cells. The protocol required incubating cells overnight in the presence of TGF- $\beta$  and assaying proliferation by measuring incorporation of tritiated thymidine (a radiolabelled nucleotide) into DNA. As expected, TGF- $\beta$  caused a dose-dependent inhibition of proliferation. Contrary to my expectations, however, a soluble preparation of parasite eggs stimulated rather than inhibited growth. For the rest of my stay I focused my attention on this phenomenon.

My investigation involved a biochemical analysis of the receptor-mediated initiation of proliferation

### My investigation involved a biochemical analysis of the receptor-mediated initiation of proliferation in epithelial cells.

in epithelial cells. It was discovered that a soluble egg extract stimulates tyrosine phosphorylation of a protein which by its appearance on SDS-PAGE appears to be MAP-kinase (Mitogen Activated Protein kinase), a well characterized intracellular messenger protein. This protein also became tyrosine-phosphorylated following exposure to mammalian recombinant EGF (Epidermal Growth Factor). Cells stimulated with EGF showed tyrosine phosphorylation of a 170 kDa protein likely to be the EGF receptor. However, in cells stimulated with soluble egg preparation phosphorylation of this protein was not seen, suggesting that the egg-derived growth factor does not operate via

the EGF receptor.

I also began work to isolate the growth factor responsible for the proliferation induced in epithelial cells, but this phase of the project could not be completed.

My stay in the lab was an invaluable experience, and removed a great deal of the mystery behind research. I met some fascinating people who have tutored me in laboratory techniques, research presentation and investigative analysis. In working with these people I obtained an appreciation of the dedication and commitment required by a successful research scientist.

The past ten weeks also included talks and seminars with professionals in academia and industry. These sessions were a significant part of the program for me, providing as they did an understanding of the processes involved in obtaining an internship or residency, working in industry, becoming an academic, and balancing a full career with life outside veterinary science. I would like to thank the Career Day counselors for their input and guidance.

My fellow Leadership students at the cooperative residency provided an environment that was extremely active, entertaining and fulfilling. Many strong friendships have emerged.

I would like to thank Dr. Pearce for his mentoring, and for maintaining an energetic and motivating work environment conducive to high productivity. A special thanks goes to Dr. Esther Racoosin for her countless hours of help and guidance, without which I would have had little to show for my hours in the lab. This experience would have been impossible were it not for the efforts of Dr. McGregor, Linda Griswold and the outstanding support of Professor David Fraser.

Thanks for a great summer.



## Michelle Dries - Sydney University



I was first introduced to research at the age of 17 when I completed a research project as part of my secondary education. During this period I became involved with veterinary researchers and have continued to explore this area during vacations from my veterinary science degree. The leadership program represented the ideal opportunity to gain valuable skills in scientific research as well as identifying possible career options.

Using ambulatory electrocardiography I investigated the effects of heart rate on the polarity change seen in the T wave of the horse with guidance from Dr. Sydney Moise and Dr. Robert Gilmour. Based on our own observations and those of others, I hypothesized that the change in polarity of the T-wave (T-wave alternans or 'flipping') in horses was not an abnormality, but instead a normal electrophysiologic response of the horse to changes in the heart rate. Moreover, I was interested in determining the mechanism for this change in polarity that is unique to the horse.

Nine horses were studied. I wanted to determine if normal horses had T-wave alternans and what RR-intervals (heart rate) were

associated with positive or negative polarity. A three channel recording of the base-apex, Y, and left precordial was used. These recordings were repeated after 3 treatments that would change the heart rate: (1) xylazine (decrease heart rate), (2) isoproterenol (increase heart rate), (3) exercise (increase heart rate followed by a slowing). Blood pressure was determined in selected horses.

Additional cellular electrophysiologic studies were performed to determine if differences in endocardial and epicardial potentials changed in vitro resulting in alternans similar to the whole animal studies. Cells were impaled with microelectrodes and stimulated with different cycle lengths before and after treatment with isoproterenol and norepinephrine.

All horses showed spontaneous changes in T-wave polarity in each of the leads. Furthermore, interventions that increased or decreased the heart rate also affected the polarity of the T-wave. The T-wave polarity was significantly associated with the heart rate (RR-interval). The T-wave was positive with faster heart rates (shorter RR-intervals) and negative with slower heart rates (longer RR-intervals). During the transition from positive to negative and vice versa, the T-wave became biphasic. The change in polarity often occurred gradually over several heart beats, rather than changing abruptly.

*In vitro* electrophysiologic studies complemented the *in vivo* results. At long pacing cycle lengths the action potential duration (APD) of both the endocardium and epicardium increased; however, the epicardial APD increased more than the endocardial APD, causing the summed potentials during repolarization to change from a more positive to a more negative potential difference. This change in polarity

was enhanced when isoproterenol or norepinephrine was added to the tissue bath.

From these results we concluded that the change in the morphology of the T-wave in the horse is a normal physiologic reflection of the heart rate. Moreover, cellular electrophysiologic studies demonstrated that repolarization in the epicardium and the endocardium was affected differentially in the horse cardiac cells as a result of changes in cycle length.

### **change in the morphology of the T-wave in the horse is a normal physiologic reflection of the heart rate.**

The question remaining: Why is the horse unique in its cardiac repolarization in response to heart rate? Differences in action of repolarization currents of the horse compared to other species may be a possibility. By determining the reason for this uniqueness we can gain a better understanding of the electrical activity of the heart. This may have relevance in the investigation of cardiac arrhythmias because many clinical arrhythmias in humans and animals are due to abnormalities in repolarization.

I am extremely grateful to Dr. McGregor and Linda Griswold whose dedication to this program made this summer's experience one of the best in my life. Thanks to Dr. Moise, Dr. Gilmour and the Student Cardiology Crew for their guidance and support throughout my stay at Cornell.

I would like to thank the Simpson Trust and the Queen's Trust for their financial support which allowed me to participate in this fantastic summer program.

## Margaret Fleischli - University of Wisconsin



I decided I wanted to be a veterinarian when I was eight years old. At the time, I was motivated by a desire to cure animals. Sometime between this early decision and my first day of veterinary school, my view of what it means to be a veterinarian changed.

After some exposure to research in the summers, I studied biochemistry at Mount Holyoke College where I became interested in the molecular basis of normal development and disease. During my first year of veterinary school at the University of Wisconsin, I realized I was drawn to questions outside the realm of the practitioner. Cornell's Leadership Program seemed a great opportunity to work on basic research in a veterinary field. I had expressed an interest in cancer biology and pathology, and was fortunate to study both with Dr. Roy Levine.

My project focused on the expression of the p53 tumor suppressor gene in two canine osteosarcoma cell lines. Canine osteosarcoma is a good model of human osteosarcoma because the diseases share a similar progression and histological basis. Understanding the etiology of canine osteosarcoma

should aid in both treatment and prevention of this disease.

The tumor suppressor gene p53 is altered in roughly half of all human cancers. The p53 protein is a transcription factor involved in cell cycle control and apoptosis. After a normal cell's DNA is damaged, wild-type p53 protein causes cell cycle arrest, partly accomplished through the induction of p21, an inhibitor of cyclin-dependent protein kinases. During this arrest, DNA repair can occur. If a transformed cell's DNA is damaged, wild-type p53 protein initiates apoptosis, a type of programmed cell death. There is a strong selection among tumor cells for those which have an inactivated p53 gene. It is not known whether p53 is mutated in canine osteosarcoma, however.

### **I studied the expression of the p53 tumor suppressor gene in two canine osteosarcoma cell lines.**

To determine whether the canine osteosarcoma cell lines CO5 and CO7 contain functioning p53 protein, RNA was isolated from control cells, and from those treated with the chemotherapeutic agent, 5-fluorouracil (5FU). Neither cell line showed significant transcriptional induction of the p53 inducible genes, p21 or mdm-2, after treatment with 5FU as compared to canine fibroblasts (CF2) containing functional p53.

As a further check of p21 induction, protein extracts from CO5 and CO7 cells were prepared and analyzed by Western blotting using an antibody against p21. Although a small induction of p21 protein was observed in both CO5 and CO7, the response was markedly less than in CF2 cells.

Taken together these results indicate that a defect exists in the p53 pathway. To further characterize the defect, p53 mRNA and protein levels were measured in both osteosarcoma cell lines and in CF2. CF2 showed a moderate level of p53 mRNA expression, while CO5 showed a very low level, and CO7 a very high level. p53 protein was not detected in either the CF2 or CO5 cells, but was very abundant in CO7.

The lack of p53 activity in CO5 may be explained by its low level of expression, perhaps due to a mutation in the promoter region of p53, or in the gene of a transcription factor. The over-expression of p53 in CO7 may indicate a mutation that stabilizes the p53 protein, as has been shown in studies of p53 mutations in other species.

To determine if there were mutations in the p53 genes of either CO5 or CO7, regions of the p53 gene, corresponding to potential mutational "hot spots," were cloned from each cell and the DNA sequenced.

Mutations were not detected in the CO5 p53 gene, whereas the CO7 p53 gene contained a GC to AA mutation in codon 273, resulting in an arginine being replaced by glutamine. The loss of arginine at this location of the p53 protein has been shown to directly inactivate p53 in many human tumors.

I thoroughly enjoyed my experiences this summer. I learned a great deal about research in molecular biology, about opportunities in research and veterinary medicine, and about people and cultures from around the world. I want to thank Dr. Roy Levine for guiding me in the lab; Dr. Douglas McGregor, Linda Griswold, and Allan Kaplan for organizing the program; Merck and NIH for funding my stay; and all of the '96 Leadership Students for wonderful memories!



## Patricia Gearhart - Michigan State University



My scientific focus this summer has been the study of MHC class I gene expression in the horse, specifically as it relates to the immunology of pregnancy. A central question being addressed by the Antczak lab is: What is the mechanism that enables a fetus to survive in the potentially hostile immunologic environment of its mother? One of the hypotheses put forth to explain this phenomenon is the relative immunologic neutrality of the conceptus. Indeed, trophoblast cells of the allantochorion are MHC class I negative, and as such, form a protective neutral "shield" around the fetus. On the other hand, the invasive trophoblasts of the chorionic girdle are MHC class I positive. In the horse, these invasive cells form the endometrial cups between days 36 to 38 of pregnancy. Interestingly, endometrial cup formation is accompanied by a proliferation of maternal lymphocytes at the site. At this time a maternal humoral immune response is mounted to the fetus. However, as the cups mature, the cup cells become class I negative.

In order to understand the precise role MHC class I expression plays during pregnancy, it is neces-

sary to understand the regulation of expression of these genes. Equine MHC class I gene products are similar to class I proteins of other species. They are 44 kDa membrane proteins whose structure consists of the alpha1, alpha 2, alpha 3, transmembrane and cytoplasmic domains. The polymorphic alpha 1 and alpha 2 domains form the peptide binding site of the class I molecule. Efforts to understand the exact structure and the expression of the equine MHC genes and their products are a current focus in the lab. The cDNA sequences for nine class 1 genes are known for the horse, and are designated A1, B1, B2, B3, B4, C1, E1, 8-9, and 1-29. The 8-9 and 1-29 cDNA clones most likely represent two distinct polymorphic loci associated with the A2 MHC haplotype. Similarly, B2 is a polymorphic allele of the A3 haplotype. A1, C1, and E1 appear to be nonpolymorphic class 1 genes encoded by distinct loci.

The delineation of the complement of genes expressed in different tissues of horses carrying known MHC haplotypes is an important first step in the study of the regulation of expression of these genes. A RT-PCR experimental approach was used in which mRNA was isolated from lymphocytes and chorionic girdle cells from horses and conceptuses with the A2/A2 and A3/A3 haplotypes. Isolation of mRNA from these cells allowed us to examine the genes being actively transcribed in these individuals. First, strand cDNA was synthesized from the mRNA template. Next, PCR primers specific for conserved regions of class I molecules were used to amplify cDNA fragments from all the class I transcripts present in the isolated mRNA. Following amplification, the PCR products were subcloned and their sequence was determined.

We accumulated sequence data

from class I transmembrane and cytoplasmic domains which confirm that there is equal expression of the 8-9 and 1-29 class I genes associated with the A2 haplotype, and a single polymorphic locus (B2) expressed by the A3 haplotype. Sequences from the polymorphic regions of these genes extend and confirm these results for the A3 haplotype. Furthermore, we found that the polymorphic loci are expressed in equal abundance in lymphocytes and chorionic girdle cells, while expression of the nonpolymorphic A1 locus is greatly reduced in the girdle compared to lymphocytes. This suggests that placenta and lymphocytes have distinct regulatory mechanisms controlling class I gene expression.

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**Polymorphic loci are expressed in equal abundance in lymphocytes and chorionic girdle cells, while expression of the nonpolymorphic A1 locus is greatly reduced in the girdle compared to lymphocytes.**

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I would like to thank Dr. Doug Antczak for making this summer possible for me, Mr. Wayne Gottlieb from whom I assumed this project, and the Dorothy Russell Havemeyer Foundation for their generous support of this research.

## Jessica Geyer - North Carolina State University



This summer I had the privilege of participating in a research project in the lab of Dr. Judith Appleton at the James A. Baker Institute of Animal Health. Dr. Appleton's research interest is in the immunology and pathogenesis of the parasitic nematode, *Trichinella spiralis*. In general, the Appleton lab studies interactions between the larvae and the host's mucosal immunity in the small intestine. The specific goal of my project was to identify factors in the intestinal environment that activate *T. spiralis* larvae to invade the intestinal epithelium.

### **The specific goal of my project was to identify factors in the intestinal environment that activate *T. spiralis* larvae to invade the intestinal epithelium.**

The basis of my research was an *in vitro* assay that provides a model for larval invasion of the small intestine. In this assay, muscle larvae invade and travel through epithelial monolayers leaving trails of dead cells. The damaged area can

be visualized by staining with trypan blue and quantified using a computer-assisted image capture system. During the development of this assay, it was discovered that larvae must be exposed to intestinal contents in order to be invasive. The question then arose: what is it about gut contents that activates the larvae? Two approaches have been proposed for investigating the presence of an "activating factor" in gut contents: one is to choose various compounds known to exist in the intestinal environment and to test them for their ability to activate larvae; the other is to fractionate gut contents in an attempt to isolate the activity in one of the fractions. My experiments utilized both of these methods.

My initial experiments tested the ability of the proteolytic enzyme trypsin and the bile acid deoxycholate to activate larvae. These substances have been reported by others to alter the behavior of larvae *in vitro*. My results indicated that trypsin and/or deoxycholate were not effective in stimulating muscle larvae to invade epithelial cells.

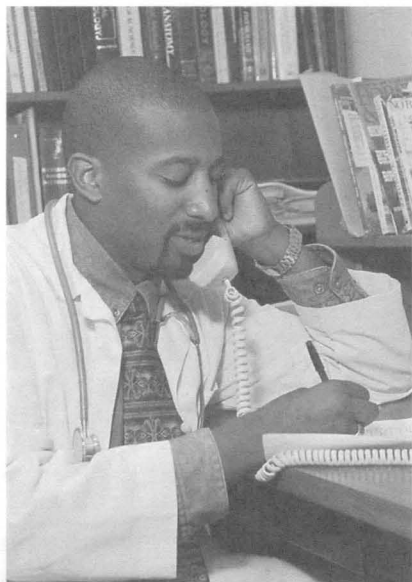
The next step involved separating gut contents into fractions based on size. Using ultrafiltration, the contents were separated into two portions: >30 and <30 kDa. When these fractions were used in the invasion assay, the <30 kDa fraction retained the ability to activate larvae. Following this separation, another similar filtration was performed that separated the gut contents into >10 and <10 kDa. The results from the assay using these fractions showed that the majority of the larval activation capacity was associated with the smaller (<10 kDa) fraction. The <10 kDa filtrate of gut contents was then treated with pepsin, in an attempt to degrade peptide components. This treatment did not alter the capacity of the filtrate to activate larvae.

I tested whole rat bile as well as sheep bile in the assay. Both effectively activated the larvae to invade. This result suggests that the activation factor is introduced into the intestinal environment via the bile.

After discovering that the activating factor was of low molecular weight, a literature search turned up a candidate molecule: vasoactive intestinal peptide, a gut hormone and neurotransmitter. A synthetic form of the peptide was tested and shown to stimulate the larvae at concentrations of  $10^{-7}$  and  $10^{-8}$  M. More research needs to be performed with this compound, but preliminary findings are intriguing.

Although I was unable to completely solve the activation factor mystery, I feel that I was able to accomplish a lot in only ten weeks. I would like to thank all the people who made this experience possible: Dr. Appleton for providing a great project; Dr. McVay and the rest of the lab for day to day assistance; Dr. Carter for urging me to participate in the program; and the USDA for providing the funds to make this an unforgettable summer.

## Jonathan Goodwin - Tuskegee University



As an undergraduate at Cornell University in 1995, I read the 1994 booklet produced by the Leadership Program for Veterinary Students. I was so excited about the program that I went to see Linda Griswold right away. She kindly informed me that I would not only have to complete at least one year in veterinary school, but would need to excel because the program is intensely competitive. I said, "Thank you very much Mrs. Griswold. I'll see you next summer." With God's help, my promise came true and I was offered a position in the 1996 Cornell Leadership Program.

This summer I was fortunate enough to work with Dr. Katherine A. Houpt in the Animal Behavior Clinic, where I was given a choice of four research projects. I was able to choose between topics ranging from observing baboon behavior for environmental enrichment studies, to studying pregnant mare behavior in reaction to stable confinement. However, I chose to do my research on the little-known subject of thunderphobic dogs.

Summer in the Finger Lakes region is often accompanied by severe thunderstorms. During this season, many dog owners experience feel-

ings of dread because they have witnessed their dog's reaction in this situation and remember the desperate fear in their dog's eyes. In some canines, the sudden booming sounds of thunder, fireworks, or gunshots elicit fearful responses. The problem may become so severe that thunder, or even the sound of rain, or a drop in the barometric pressure that accompanies thunderstorms, may stimulate a fearful response.

I selected ten dogs for my study. Each was assigned a numeric value designating severity of fear (0=no response 7=psychological shock). Then I randomly assigned each dog to one of two drug protocols in an effort to compare their efficacy. The first protocol was a combination of the barbiturate, phenobarbital, given two times each day and the beta-blocker propranolol given three times a day throughout the summer. The second protocol consisted of the benzodiazepine, clorazepate dipotassium, also known as Tranxene-sd (sustained delivery), which was given only on the days when thunderstorms were predicted to occur.

### **Tranxene-sd was the superior anxiolytic drug for this group of canines with noise phobias.**

As the study continued, it became clear that Tranxene-sd was the superior anxiolytic drug for this group of canines with noise phobias. Although, we had some success with the phenobarbital and propranolol combination, the dogs also experienced many side effects with the drugs such as vomiting, nausea, and ataxia. Nevertheless, we determined that while neither drug was a cure alone it was a step in the right direction, especially if

accompanied by a counter-conditioning behavior modification program.

This summer has been a truly enriching experience. I would advise any veterinary student who is thinking about a career in research to apply. I would not have been as successful this summer, however, if it hadn't been for the help of others. First and foremost, I want to thank my Lord and Savior Jesus Christ for blessing me with the strength and ability to get to this point in my life. I also want to thank the NIH and the Robert W. Woodruff Foundation for their generosity in providing my fellowship, and Dr. Katherine A. Houpt and Mrs. Linda Griswold for their endearing spirits and confidence in my abilities. I would be remiss to forget all the support I received in my research from Justine Swaney, Heather Houseman, Kevin A.R.A. Kunkel, Noelina Arcinigas, and especially Jacqueline Martinez, who without her love and support, I could not have made it through this challenging summer.

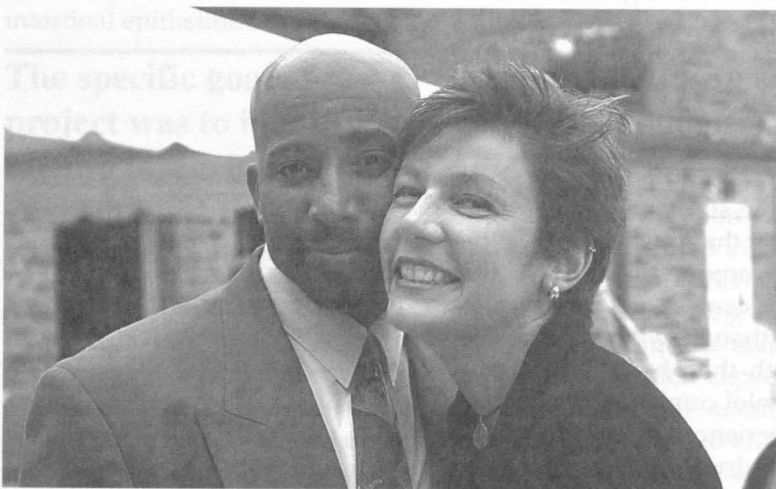




Allison Stewart and her two mentors, Dr. Robin Gleed and Dr. Barry Cooper.



Dr. Donald Smith chatting with Amy Schein



Jonathan Goodwin and Suzanne McNabb

## Tamara Gull - Tufts University



A primary aim of the research conducted at the Equine Genetics Center is to elucidate the immunologic relationship between dam and fetus during pregnancy. One approach to this research is to create interspecies hybrids between horse and donkey, thereby increasing the genetic disparity between dam and fetus. Toward this goal, my project strove to answer the following questions: how polymorphic are donkey Major Histocompatibility Complex (MHC) class I antigens? Do donkeys carrying donkey fetuses and mares carrying mule fetuses mount immune responses against their offspring during pregnancy? Which fetal antigens are recognized in interspecies mule pregnancies? Much is known about pregnancy in horses, but other members of the genus *Equus* have not been so well studied.

The first goal of this project was to investigate the polymorphism present in donkey MHC class I cell surface antigens, which are expressed by the fetus in early pregnancy. Antibody-positive sera were surveyed for reactivity to lymphocytes from all of the donkeys, mules and hinnies at the Center. Lymphocytes from horse parents of mules

(jack x mare) and hinnies (stallion x jennet) were also assayed to determine how much, if any, horse cross-reactivity was present in the sera. Three clear donkey-specific lymphocyte antigens were identified, with a suggestion of possibly two other donkey antigens which show some cross-reactivity. There is also at least one other donkey antigen present for which we have no antibody. It was demonstrated in several hybrids which failed to react with any of our antisera.

An interesting result of this study is the suggestion that MHC polymorphism in donkeys may be limited. This is in contrast to the horse, in which at least twenty different MHC class I haplotypes exist.

The next questions were examined using a microcytotoxicity assay in which sera from pregnant jennets and mares carrying mule pregnancies were mixed with lymphocytes from the jack donkey sires of the pregnancies. The presence of antibody was assayed by measuring the percentage of lymphocytes killed. Positive sera were titrated at days 20, 40, 80 and 120 of pregnancy. While virtually all animals tested exhibited some level of antibody production, there were differences between donkeys, mares carrying mules, and horse-only pregnancies. These results suggest that the maternal immune response is weaker and occurs later in both donkey pregnancies and in mares carrying mule pregnancies than in mares carrying horse pregnancies. We were unable to characterize a known primary immune response due to the complicated reproductive histories of animals in the herd. Secondary immune responses were also difficult to characterize, because it is not known if the jack donkeys that sired the pregnancies contributed the same or different MHC class I haplotypes to each conceptus they

sired.

Finally, this study sought to discover which fetal antigens are recognized by mares carrying mule pregnancies. It was unknown whether the maternal immune response was directed against antigens specific to individual donkeys or against an antigen common to all donkeys. Our experiments showed that antibody from each mare was cytotoxic to only some of the donkeys and mules tested, demonstrating that a single common donkey antigen was not the target of the immune response. In fact, many of the sera formed clusters of identical specificity to those found in donkey alloantisera. This clustering process was used with sera from intraspecies horse pregnancy to define the MHC class I antigens of horses. A strong possibility exists, therefore, that the polymorphic regions of donkey MHC class I molecules are those being recognized by the mare during mule pregnancies.

I would like to thank Dr. Doug Antczak of the Baker Institute for Animal Health and the Equine Genetics Center for his invaluable assistance this summer. I would also like to extend my appreciation to Mr. Gene Pranzo, the Dorothy Russell Havemeyer Foundation and the National Institutes of Health for their generous support of this research.

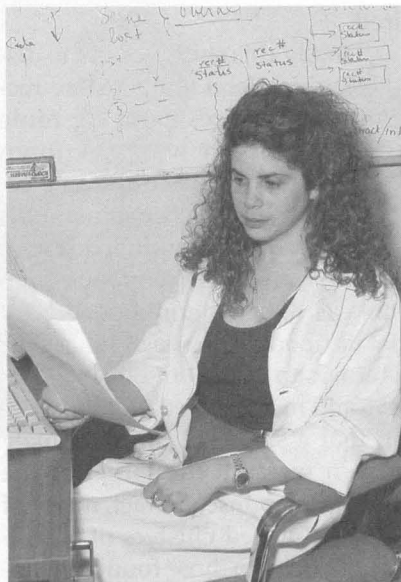
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**A strong possibility exists that polymorphic regions of donkey MHC class I molecules are recognized by the mare during mule pregnancies.**

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## Jennifer Hess - Cornell University



What is the etiology of feline dental cervical line lesions?

I am a 1992 graduate of the College of Arts and Sciences at Cornell University in Biology. The Leadership Program continues my participation in science which began as an undergraduate and enabled me to pursue projects in the fields of plant genetics, *Drosophila* genetics, and human genetics. I am extremely grateful for my experience this summer working with Dr. Janet Scarlett on Small Animal Epidemiology. Our project was an investigation of the etiology of feline dental cervical line lesions.

Feline Cervical line lesions (CLL) have many different names in the literature: external osteoclastic resorption lesions, idiopathic buccocervical erosion, chronic subgingival tooth erosion. The variety of names reflects the variety of hypothesis proposed to account for their formation. "Idiopathic" conveys the nebulous etiology of this oral pathology.

What are cervical line lesions? Originally they were considered to be the caries of cats (Eisenmenger 1985), which they are not. Caries are defined as: the demineralization of tooth enamel by toxins produced by

carbohydrate-fermenting bacteria. Conversely, cervical line lesions are a type of external resorption. External resorption results from the odontoclastic activity of the periosteum of the alveolar bone (Harvey 1985). Cervical line lesions begin at the junction between the crown and the root, then progress both into the crown and the root. This is a type of bone or cementum remodeling process which repeats itself until ankylosis occurs and the tooth is permanent. This mirrors the clinical observation that once the CLL appears, little can be done to stop its progress.

The history of CLL indicates that it is a recent disease. An examination of skulls from cats in 1935 failed to reveal cervical line lesions. In 1976, Schenk first brought CLL into the focus of veterinary medicine. So, the disease must have appeared somewhere between 1935 and the 1970's when CLL became a topic of importance to feline health. One must ask: what has changed for cats, especially in the last 20-30 years?

The hypothesis as to the origin of CLL follow four subsets: nutritional, viral, environmental, or hormonal. These four subsets are not mutually exclusive and interplay between several factors must be considered. Nutritional factors are the focus of our pilot study. It has been suggested that the addition of urinary acidifiers to feline diets to promote sound feline urinary health may have the complication of lowering the pH of the saliva. Preliminary studies indicate that this is not correct. Other studies indicate that raw liver diets are associated with increased frequency of CLL. Some researchers hypothesize that nutritional hyperparathyroidism initiates the resorption process of the odontoclasts. Another hypothesis is that there is a calcium deficiency in the diet or a calcium-phosphorus

imbalance. The viral hypothesis first considered FeLv but it was quickly dismissed as a candidate. The calicivirus was isolated from a set of cats with CLL but one must consider the ubiquitous presence of the calicivirus (an URV) in cats.

Other environmental factors postulated to play a role in CLL development are the presence of calculus and gingivitis. In addition, chronic regurgitation may injure enamel, causing resorption as it does in human bulemics.

My participation in the project consisted of creating coding manuals, encoding data (using Paradox program), verifying data, and analyzing data (using Quattropro, Statistix, and Systat programs). The results of this pilot study revealed that the time a cat spent outdoors was a negative indicator for developing cervical line lesions. We found no association between cats with CLL and any known virus, type of diet, hairballs/vomiting, or a particular type of human food treat.

Many thanks to Dr. Janet Scarlett, Dr. John Saidla, and Nancy Gift for their patient and enthusiastic support of my endeavors. Thanks also to NIH and Merck for my summer support.

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**We found no association between cats with CLL and any known virus, type of diet, hairballs/vomiting, or a particular type of human food treat.**

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## Sarina Hinsley - Cornell University



This Summer I was sponsored by NIH and Merck to research structure-function relationships of the nicotinic acetylcholine receptor (nAChR) through single channel recordings. Working in Dr. Oswald's lab in the Department of Pharmacology, I utilized the technique of patch-clamping to explore the effects of different cholinergic agonists on current through the nAChR. Analysis of this and future data will be used to reveal structural details of the nAChR.

### **I utilized the technique of patch-clamping to explore the effects of different cholinergic agonists on current through the nAChR.**

The nAChR is an example of a ligand-gated ion channel. Two classes of this receptor are known: the muscle type and the neuronal type. We investigated the muscle type which is comprised of four different subunits in the following combination:  $\alpha_2\beta\gamma\delta$ . Each subunit within the pentameric receptor traverses the cell membrane four

times. These membrane spanning regions (M1-M4) are believed to be  $\alpha$  helices and the M2 region of each subunit comes together to line the pore through which the permeant cations travel when an agonist binds to the receptor.

Under physiologic conditions, two molecules of acetylcholine, a neurotransmitter, bind to each of the  $\alpha$  subunits and a conformational shift within the pore of the receptor occurs transmitting the binding into an opening of the ion channel. In the case of the neuromuscular junction, this results in transmission of a signal from a nerve cell onto a muscle fiber and, through a series of electrical and biochemical events, into a muscular contraction.

Continuing previous work, we used synthetic agonists to stimulate and study the nAChR that are conveniently expressed in a cell culture line derived from a mouse tumor. Acetylcholine, synthesized at the nerve terminus, has both an ester bond as well as a quaternary ammonium ion. These two functional groups are separated by 5.9 Å. 1,1 dimethyl-4-acetylpiperazinium iodide (PIP) is the basic agonist used in probing the nAChR. The synthetic agonists mimic this structure and function as well as allow for research flexibility by the variations of R groups added to the carbonyl group. By changing the R group, we are adjusting the size and hydrophobicity of the agonist and thus how far it will penetrate the ion channel.

We created tiny pipets from borosilicate capillary tubes and filled these with a known concentration of the agonist we wished to study. Then, we inserted a wire electrode into the pipet and fixed it onto a manipulator. We applied positive pressure to the pipet and lowered it into a bath containing the cells to be studied. A ground was also in the bath. Using a microscope

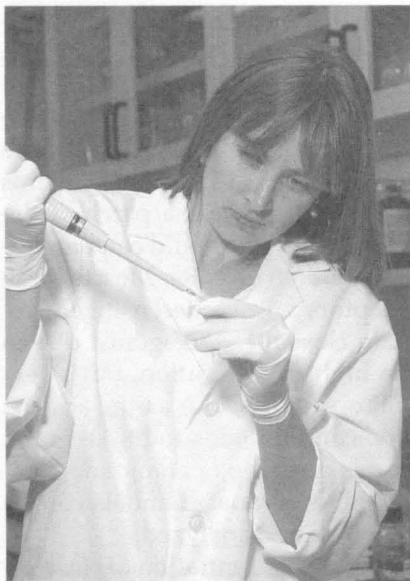
to visually guide the pipet, we directed the pipet to the surface of a cell. Upon contact with the cell membrane, we applied negative pressure to create a high resistance seal to the cell membrane. With this action, we isolated a patch of the membrane that should contain several hundred nAChRs. Most of these receptors will be desensitized (remain closed) to the agonist due to the high concentration, but a few will open and close as the agonist binds and unbinds to the receptor. In this way, the opening and closing of individual channels can be observed and analyzed.

If the concentration of the chosen agonist is high enough, two agonist molecules will activate that channel and a third molecule will enter the pore and prevent ions from flowing into the cell. This is called blockade. By varying the agonist's R group, the size and hydrophobicity of the agonist can be altered. This will be reflected in the currents and blockade seen in the single channel recordings because the more hydrophobic the agonist, the more tightly it will be bound to the hydrophobic regions of the pore. Therefore, by comparing the blockade events for the different agonists, structural information can be inferred from ion movement through the receptor protein.

This summer, we used 1  $\mu$ M concentrations of several agonists in an effort to stimulate a single channel within the patch. After we obtain single channel recordings, we will begin to analyze our data.

I would like to thank Dr. Oswald, Dr. Kornreich, Dr. McGregor and Linda Griswold for all of their help in making this a wonderful experience.

## Antonia Jameson - Cornell University



When I decided to switch my career from publishing to veterinary medicine, I had every intention of going into small animal practice. But as I began studying biology in preparation for vet school, I became fascinated by cell biology and the molecular basis of disease. By the time I came to Cornell, I was eager to get experience in a laboratory. I was delighted to be accepted into the Leadership Program because it gave me a long-hoped-for opportunity to do research. Moreover, I was placed on a project on signal transduction, a subject of particular interest to me.

This summer, I worked in Prof. Rick Cerione's laboratory on the characterization of "Cool", a recently isolated protein that is part of a signalling pathway implicated in cytokinesis and cell-cycle progression. Cells have evolved elaborate mechanisms to transmit external signals from the plasma membrane to the nucleus, where they influence gene expression and thus cell behavior. These consist of cascades of protein-protein interactions and typically include receptors, guanine nucleotide-binding proteins (G proteins), regulatory proteins, protein kinases, and transcrip-

tion factors. Many of the signalling proteins, if mutated, can become oncogenic. The G proteins play a particularly critical role: they function as physiological switches, cycling between an active GTP-bound state and an inactive GDP-bound state. Under normal conditions, they are turned on by regulatory proteins (that stimulates the exchange of GTP for GDP) in response to an external signal. These activation events then allow the G proteins to initiate signaling cascades that often lead to the nucleus.

Cool (for "cloned out of library") was discovered by Dr. Shubha Bagrodia, under whose supervision I worked, in a search for proteins that interact with PAK, a serine-threonine kinase activated by the CDC42 G protein. A yeast two-hybrid assay was performed, and a partial cDNA of Cool was isolated and sequenced. The sequence shows that Cool has three domains found in other proteins: a src homology domain (SH3), a dbl homology domain (DH), and a plextrin homology domain (PH). The SH3 domain is believed to mediate binding to PAK, while the DH domain in tandem with the PH domain, places Cool in a family of guanine-nucleotide exchange factors that directly activate G proteins.

My project focused on the interaction of Cool with PAK. We wanted to see if disrupting the association of the two proteins would have any effect on cells. A version of Cool with a point mutation changing a tryptophan to a lysine in the SH3 domain was made via the polymerase chain reaction (PCR). A primer with the desired change was used to generate a short gene fragment. This product was then used as a primer in a second PCR reaction to generate a longer fragment with unique restriction enzyme sites at either end. This piece of DNA was digested with BamHI and

EcoRI and ligated into appropriate plasmid vectors. The plasmids were introduced into *E. coli* by electroporation. DNA from the bacteria was isolated, digested with restriction enzymes, and separated by gel electrophoresis to verify that the desired constructs had been made.

**My project focused on the interaction of Cool with PAK. We wanted to see if disrupting the association of the two proteins would have any effect on cells.**

Before transfecting mammalian cells with mutated and normal versions of Cool, the interaction of Cool and PAK was tested in vitro. Bacteria were induced with IPTG to express a fusion protein consisting of glutathione-S-transferase linked to the SH3 domain of Cool. The protein was purified by incubation with glutathione-agarose beads. The beads were then exposed to lysates from cells transfected with PAK. After incubation, the cells were treated with SDS to release the Cool fusion protein, which was then run on an SDS-polyacrylamide gel and transferred to a membrane for a Western blot. The membrane was probed with antibodies to PAK to see if it had bound to Cool. As this is being written, I am awaiting the results of the probe.

The last ten weeks have been a wonderful experience: I learned a variety of techniques used in molecular biology and I got a sense of how scientists approach a new problem. I would like to thank the many who have helped me: the NIH and the Merck Foundation, the organizers of the Leadership Program, Rick Cerione and the people of his lab. I am especially grateful to Shubha Bagrodia for her patience and generosity.



## Bernice Mangnall - Massey University



I am almost at the end of my final year at Massey University in New Zealand. Prior to studying veterinary science I obtained a Bachelor of Agricultural Science (Hons) from Lincoln University (NZ). It has always been my goal to be a veterinarian and to be actively involved in the New Zealand agricultural industry. I want to do this in a field advisory/research type role.

I have spent the summer in Dr. Roger Avery and Dr. Peggy Barr's laboratory studying feline immunodeficiency virus (FIV). FIV is a retrovirus which has RNA as its genetic material and replicates using a process known as 'reverse transcription'. FIV is therefore a parasite of DNA based organisms as it uses the host cell's machinery to replicate itself. FIV belongs to the subfamily Lentivirinae (meaning 'slow moving'). It causes progressive immunosuppression with clinical signs occurring many years after initial infection.

Previous research has shown that cats inoculated with proviral DNA (naked DNA) of FIV-PPR (an infectious clone of domestic cat FIV) resulted in infection and seroconversion of domestic cats. My project aimed to repeat this

result with FIV-PPR, and then test our FIV clones (Oma-1 and Oma-2) to see if they too could cause a response, ie, viraemia or sero-conversion.

### **My project aimed to test FIV clones (Oma-1 and Oma-2) to see if they too could cause a viraemia or sero-conversion**

Eight cats were used in the experiment. Each of these was allocated to one of four treatment groups as follows: 1) Control - pBlueScript plasmid without viral DNA; 2) pFIV-PPR - an infectious clone of domestic cat FIV; 3) pFIV-Oma1 - a clone of Pallas cat FIV which is non-infectious in tissue culture; and 4) pFIV-Oma2 - a clone of Pallas cat FIV which is infectious in lymphocytes and Crandell Feline Kidney (CRFK) cells.

The proviral DNA was grown as plasmids in DH5 (*E. coli*) and isolated using a commercial Qiagen kit which removed any endotoxins from the DNA preparation. 200ug of proviral DNA was inoculated intramuscularly into the right hind leg of each cat according to its treatment group.

Weekly blood samples were taken to detect viraemia or an immune response (production of antibody). Lymphocytes were isolated and cultured to detect 'reverse transcriptase' activity (which is indicative of virus replication), and also to detect a reduction in lymphocyte number which indicates the cytopathic effect of FIV. Polymerase chain reaction (PCR) was used to amplify viral DNA in the host lymphocytes. Plasma samples were used to detect the presence of antibody using the Western blot technique.

Unfortunately ten weeks was not enough time as I had delays in

isolating enough DNA for inoculation of the cats. At the time of my departure one of the pFIV-PPR cats was positive for antibodies to FIV on the Western Blot test. This animal also had slightly elevated reverse transcriptase levels which will likely increase within a few weeks. PCR results for the same animal and for the two pFIV-Oma1 cats showed bright bands in the target 800 base pair region but this must be repeated as the positive control did not work.

Ultimately the ideal result would be to find evidence that inoculation with naked DNA can elicit an immune response without causing an infectious viraemia. This might be accomplished using a non-infectious clone, such as pFIV-Oma1, which would not produce an infectious viraemia but may be able to induce an immune response great enough to protect the cat against the homologous FIV strain. Similar proviral DNA clones could then be developed for domestic cat strains, leading to the potential development of a vaccine against domestic cat FIV.

I found this summer to be a great experience - not only with respect to research but also in the many new life long friends that I have made. I would especially like to thank the Gould Foundation for sponsoring me. I also wish to thank my mentors Dr. Roger Avery and Dr. Peggy Barr, and my fellow "lab rats" Wendy Hoose, Janny, Marcia, Lily and Fan. It would have not been possible for me to take seven weeks off my studies in New Zealand without the support of my Dean (Prof. Stockdale), lecturers, and classmates (especially Lyndsay who has taken notes for me for the many lectures I will have to catch up on!).

## Suzanne McNabb - University of Queensland



When I was very young, I decided I wanted to be a veterinarian. This plan was somewhat disrupted by high school work experience during which I saw a disheartened and disillusioned veterinarian in practice. After leaving school, I completed a Bachelor of Science degree with a double major in pathology and pharmacology at The University of Melbourne, Australia. I was planning to follow a research pathway, starting with a Ph.D., when I remembered my original desire to be a Vet. I am currently in my final year of the Bachelor of Veterinary Science program at The University of Queensland. I have spent the summer in Dr. Schat's laboratory in the Department of Microbiology, working on Marek's Disease Virus (MDV).

MDV is an alpha-herpesvirus which occurs in the poultry industry worldwide. Its clinical manifestation, Marek's Disease (MD), produces fatal lymphomas. Marek's disease has enormous economic repercussions. Control of MD is predominantly by vaccination. Although reasonably effective, new, very virulent strains of MDV have created the need for improved vaccines.

Recombinant viruses provide a new direction for vaccine development. My research aimed to create a recombinant Fowl Pox Virus (rFPV) containing the ICP4 protein of MDV. ICP4 is an immediate early protein of herpesvirus which is recognised by cytotoxic T lymphocytes (CTL). It activates many genes involved in virus replication and thus has an important role in latency and transformation. ICP4 is required in FPV in order to ensure that the CTL response to MDV challenge is ICP4 specific.

The experimental design involved using restriction enzyme digestion (RE digestion) of DNA from a series of plasmids to acquire the necessary promoters, markers (lacZ) and multiple cloning sites (MCS). The ICP4 fragment was released from the plasmid pmd145, and the Bluescript plasmid was linearized with *Bam*H1 RE digestion. The ICP4 fragment and the linear vector were ligated to produce a circular plasmid. Successful transformation with *E.Coli* DH5 $\alpha$  yielded white colonies, where the lacZ region of pBS had been disrupted by the insertion of ICP4. Site directed mutagenesis is then needed to remove a *Bgl* II restriction enzyme site from ICP4 before RE digestion can be used. A three vector system is used to create a plasmid containing both ICP4 and lacZ, both with promoters. Blue plaques produced from transfection of FPV infected chicken embryo fibroblast cells with the plasmid, are then rFPV clones which may be isolated.

The following methods were involved: DNA extraction of pBS and pmd145; Restriction enzyme digestion with *Bam*H1; Phenol/Chloroform precipitation of *Bam*H1 digested pBS; Gel purification of ICP4 fragment; Dephosphorylation of pBS; Ligation of pBS and ICP4, and Transformation using *E.coli* DH5  $\alpha$  competent cells plated onto

Xgal and IPTG treated LB/ampicillin plates.

Seven ligations and eleven transformations were performed with only three producing white colonies. RE digestion with *Bam*H1 and *Xba*1 on the first two of these, showed them to be something other than clones (most probably lacZ mutants). In the third group of white colonies, RE digestion with *Bam*H1 produced two bands on gel electrophoresis, indicating that a clone had been produced. Therefore, the first step in the process of creating the rFPV was completed! Although I did not produce the rFPV myself, the experience in molecular biology and learning specific techniques was challenging, interesting and enjoyable. The rFPV project will now be continued by other members of Dr. Schat's team.

Dr. Schat has been an excellent mentor in all respects. He has provided me with much practical information, and with many topics for thought and discussion, relating to everything from avian virology to work as a third-world veterinarian. Assistance, patience and humour from the other members of the laboratory, most especially Priscilla O'Connell, has also been greatly appreciated.

I would like to thank Dr. McGregor enormously for giving me the opportunity to be involved in this program, and thanks to Linda Griswold for her organizational expertise. Finally, a special mention of the other 23 students on the program; we have been through a surprisingly diverse range of experiences and emotions in these 10 weeks and it has been for the most part an absolute ball. So, thanks for the summer and hope to see you all in Australia for the reunion!



## Polly Peterson - Washington State University



As an undergraduate studying cell and molecular biology, I was introduced to research both at school and in the laboratory jobs I held. While focusing on preparing for veterinary school, however, I found it difficult to gain the research training necessary to decide if this was a field that suited my interests. I was happy to be accepted into the Leadership Program, therefore, to both solidify earlier exposures to research and to open my eyes to the many options I had not previously considered for my career direction.

I was fortunate to be placed with the community of scientists at the James A. Baker Institute of Animal Health, specifically in the laboratory of Dr. Colin Parrish. It was an honor to discover that I would be studying aspects of canine parvovirus infection with a highly esteemed group of researchers who are famous for their discovery of this and other infectious diseases.

Dr. Parrish directed me in the study of aspects of parvovirus infection in polarized epithelial cells. Epithelial cells line the external and internal surfaces of the tissues of higher eukaryotes and present a primary barrier to viral infection. A thorough understanding of polar-

ized entry and release of viral antigens may be of significance to the disease pathogenesis of canine parvovirus, and might also be relevant to other viral systems.

I needed to complete several preparatory steps before attacking my specific research questions. After learning cell culture techniques and viral growth and titration procedures, I prepared a MDCK cell line that constitutively expressed the parvovirus proteins VP1 and VP2. This process involved amplifying and detecting the VP1/VP2 plasmid in an *E. coli* bacterial host, then transfecting cells with the plasmid DNA. Following growth in a selective medium, VP1/VP2 positive cells were identified using immunofluorescence. Finally, positive cells were grown from single cell clones into cultures of cells that could be used for later experiments.

Next it was necessary to begin work with the polarized MDCK cells (a cell line derived from the distal nephron of a canine kidney) on permeable supports. Permeable supports permit growth medium access to both the apical and basolateral sides of the MDCK cells, but tight junctions between the cells prevent the mixing of the two media. It is possible, therefore, to grow a confluent layer of MDCK cells and then infect the monolayer exclusively from the apical or basolateral sides. Cells were inoculated on either the apical or basolateral surface with canine parvovirus and vesicular stomatitis virus, a control virus reported to infect primarily via the basolateral membrane. Cells were then washed, trypsinized from the permeable support, permitted to divide, and stained using immunoperoxidase methods.

Infected cells were counted under a light microscope and the apical and basolateral inoculations were compared. Both apically and basolaterally inoculated cells be-

came infected; however, a significantly higher proportion of cells became infected via the basolateral membrane.

### **a significantly higher proportion of cells became infected via the basolateral membrane.**

Polarized MDCK cells transfected with VP1 and VP2 were stained using immunofluorescence on either the apical or basolateral surface to determine if viral proteins are preferentially secreted to either membrane. Cells were examined under a confocal laser microscope for careful comparison, but a significant difference between the staining intensity of the two surfaces was not observed. Cells were permeabilized and the highest proportion of VP1 and VP2 was found to have remained inside the cells.

I would like to thank the NIH and the Gould Foundation for my sponsorship, and Dr. McGregor, Linda Griswold, and Allan Kaplan for arranging my summer activities. I also am greatly indebted to everyone in the Parrish lab, especially Colin Parrish, Wendy Weichert, Martha Harding and John Parker for their expertise and patience in teaching and advising me through my experiments. Finally, I would like to thank Stacy Pritt, a past participant in the program, for introducing me to this opportunity.

## Amy Schein - Cornell University



During my third year of study for a bachelor of arts degree in English literature, various influences rekindled my childhood desire to become a veterinarian. Even knowing that I would eventually like to teach gave me a huge range of choices. Prior to my ten weeks in the 1996 Leadership Program, I had no experience working in a research laboratory. During the program I learned and practiced molecular biological techniques. The program has been especially helpful for me, as it is tailored not only to research, but to introducing students to various types of research and alternative career paths available to veterinarians.

This summer I worked in Dr. Bendicht U. Pauli's lab in the Department of Pathology. For the past several years, he and his associates have been studying molecular events that occur between metastatic melanoma cells and endothelial cells of the lung. Bovine aortic endothelial cells which express the lung endothelial cell adhesion molecule Lu ECAM-1 are being used as a model system. Lu ECAM-1 promotes binding of melanoma metastases to these cells. Identification, isolation, cloning and sequencing of

the bovine Lu ECAM-1 has been achieved, revealing a 90 kDa integral membrane glycoprotein with four putative membrane spanning regions.

My project involved isolating and sequencing the human homologue of the bovine cDNA sequence. To achieve this goal a human lung cDNA library representing all sequences transcribed in the lung was screened. Plaque hybridization was performed using the known bovine Lu ECAM-1 sequence as a probe. High frequency lysis bacteria were grown and infected with  $\lambda$ gt-10 phage containing random inserts of human lung cDNA sequences. The plates were incubated for 6 to 8 hours, during which time the phage lysed the bacteria, creating plaques on the plates.

### My project involved isolating and sequencing the human homologue of the bovine Lu ECAM-1.

The plaques were lifted on to nylon membranes, which were then hybridized to the bovine Lu ECAM-1 sequence labelled with  $^{32}\text{P}$ . Due to expected sequence differences between species, low stringency conditions were used for the screenings. Plaques showing positive hybridization appeared as small black spots on an autoradiograph. The respective clones were isolated and secondary screening performed to purify and isolate them. The procedure yielded one positive clone, designated CHL-1 (first clone from human lung cDNA library). After sequencing this 1.2 Kb clone, a region of 210 nucleotides with 69% homology to Lu ECAM-1 was found.

Based on this study, and the absence of a clear signal on a human lung northern blot, we concluded

that the expression of Lu ECAM-1 in humans is lower than anticipated. Therefore, an alternative approach was chosen to isolate the human homologue of Lu ECAM-1. It involved screening a human genomic library, again using plaque hybridization technique to find the gene encoding Lu ECAM-1. We obtained two clones (4Kb and 6Kb in length) which, after sequencing, exhibited a gene with 76% homology to virtually the entire sequence of Lu ECAM-1. After further sequencing of the sequence adjacent to the 5' end of the 4kb fragment is accomplished, gene specific primers will be generated to determine the final mRNA sequence of LuECAM-1 by means of highly sensitive PCR. Dr. Pauli's team is continuing to work on this project.

Once the murine and human homologues of Lu ECAM-1 are characterized completely, both proteins will be expressed in vitro and studies for further characterization of the adhesion events between Lu ECAM-1 and melanoma cells will be performed. Knowledge regarding the molecular adhesion mechanisms can then be used to determine its role as a cellular adhesion molecule in cancer metastasis.

I learned a lot from my experience and had a great summer as well. I would especially like to express my appreciation to the NIH and the Robert W. Woodruff Foundation which funded my research this summer, as well as Dr. Pauli and Dr. Achim Gruber, who ensured that I thought about each step before doing anything. Many thanks to everyone else in the lab for their patience and sense of humor, and to Dr. McGregor and Linda Griswold for their enthusiasm and dedication to this program.



Mark Doherty, Ralph Senften-Rupp, Edwin van Duijnhoven, Jonathan Goodwin, Constantin Von der Heyden, and John Stein.



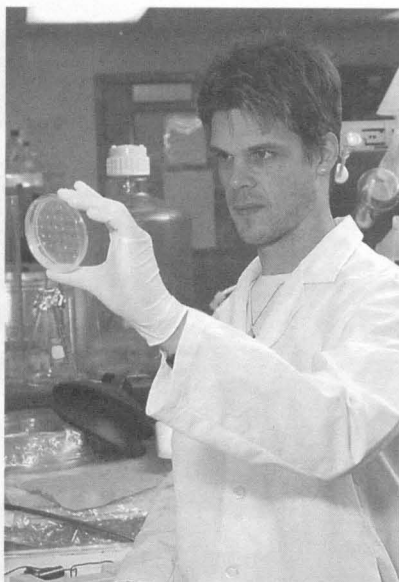
Dr. Jharna Ray and Ralph Senften-Rupp.



Mark Doherty and Michelle Dries with guests, Donald and Christopher Rickard.



## Ralph Senften-Rupp, University of Bern



I applied for admission to the Leadership Program while I was in my third year of veterinary school at the University of Berne. I did so because I am considering an academic career. Gaining insight into research has been my main goal. My mentor, Dr. J. Ray, enabled me to realize that objective this summer. I derived much benefit from her supervision and experience. Although she can be pretty tough and ambitious, she never loses her sense for humor. I was challenged by my work, but at the same time spent a wonderful and exciting time in Dr. Ray's lab. What I appreciated most, was her frankness and honesty.

Dr. Ray's lab is investigating the genetic basics of different mucopolysaccharidoses (MPS) in animals. The main goal is to establish animal models that can facilitate studies of the pathogenesis and possible therapeutic approaches to MPS-diseases. My project involved the study of MPS VI in the feline model.

MPS VI is an inherited lysosomal storage disease resulting from deficiency of the lysosomal enzyme arylsulfatase B (ASB). ASB is responsible for degrading dermatan sulfate (DS), a glycosaminoglycan

(GAG). GAGs are present as proteoglycans in the extracellular matrix. Enzyme deficiency results in the accumulation of incompletely degraded derivators sulfate (DS) in lysosomes causing disease manifestations throughout the body. Amongst these are severe bone deformities.

To understand the pathogenesis of MPS in bone and cartilage, use is made of cultured chondrocytes. These have been grown from the rib cartilage of normal and MPS VI affected cats. By measuring the ASB activity in cultured MPS VI chondrocytes, and comparing it with the level in normal chondrocytes we showed that the cells maintain the disease phenotype in culture: they have only 10-15% of the ASB-activity of normal chondrocytes, whereas other lysosomal enzymes show normal activities.

Since ASB is responsible for degrading GAGs - especially DS - we wanted to determine whether the MPS VI chondrocytes accumulate DS. To this end, we metabolically labeled the chondrocytes with  $^{35}\text{SO}_4$ , isolated the GAGs from the cell layer, and counted the radioactivity (disintegration/min/mg protein). Thereafter we separated the GAGs by electrophoresis and quantified its profile by radiophotometry. The radioactivity increase resulting from the accumulation of DS in MPS VI chondrocytes was 2.2 fold! To establish whether cultured chondrocytes maintain the biological properties of cartilage, we endeavored to detect expression of Type II Collagen (Col2A1). Col2A1 is the principal collagenous component of cartilage; it supports chondrocyte adhesion and influences the cell's differentiated phenotype.

To detect Col2A1 expression we used reverse transcription (RT)-polymerase chain reaction (PCR) of chondrocyte mRNA. Since feline

Col2A1 cDNA is not available, three primer sets were designed from the human sequence. By RT-PCR we obtained the right size fragments. By cloning each fragment using a TA cloning vector, we confirmed that the fragments are derived from Col2A1. We obtained three clones from primer set 1 and two clones from primer set 2. We did not obtain any colonies from primer set 3, that's why we sequenced the RT-PCR product directly. The sequence matched closely with human Col2A1.

From these studies we concluded: 1) that cultured feline chondrocytes maintain their differentiated state in vitro; 2) that MPS VI chondrocytes in culture maintain the disease phenotype, and 3) that these chondrocytes accumulate the GAG dermatan sulfate.

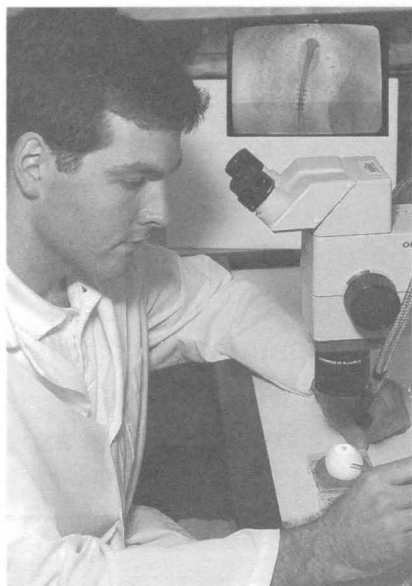
I wish to express my thanks to all the people that maintain this program. Special thanks to Dr. J. Ray and her staff (V. Scarpino, M. Verdugo, F. Du) and to Prof. G. Aguirre and his staff for their advice and friendship and to the Robert W. Woodruff Foundation for my fellowship.

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## John Stein - VA-MD Regional College of Veterinary Medicine



As a second year student at the Virginia-Maryland Regional College of Veterinary Medicine interested in pursuing postgraduate education, I was excited to participate in the 1996 Leadership Program at Cornell. Previously, I had assisted Dr. Anne Zajac in clinical parasitology research trials at Virginia-Maryland. I felt that the Leadership Program would provide an excellent opportunity to further explore the many career options available to veterinarians beyond the traditional role of private practitioner.

As a Mellon Foundation Fellow, I had the pleasure of working in Dr. Drew Noden's laboratory in the Anatomy Department. I collaborated with Dr. Darrell Evans, a post-doc in the lab, on a project that asked whether myoblasts taken from fetal and mature avian muscles retain competence to express characteristics of immature myoblasts when grafted into young embryos.

During skeletal myogenesis, uncommitted cells within paraxial mesoderm differentiate into proliferating myoblasts. Subsequently, these myoblasts migrate into peripheral locations where they form multinucleated primary myotubes

and, later, secondary myotubes. These two populations of myotubes, as well as satellite cells, constitute the definitive muscle tissue of the adult. My three specific aims were to: 1) optimize conditions for isolating quail myoblasts from muscles of different ages; 2) assess the viability of these cells in vitro, and 3) examine the behavior of these cells following transplantation into young chick embryos.

Leg muscles from quail embryos of different ages were dissected to provide the source of donor cells for transplantation. Muscle tissue was dissected, dissociated in 0.1% trypsin for 15 minutes at 37°C, washed, sieved to enrich for single cells and placed in culture dishes containing DMEM with 10% FCS.

Exploiting the greater propensity of fibroblasts to adhere to the culture dish, we developed a protocol for panning the cells to increase the ratio of myoblasts to fibroblasts. The cell suspension was cultured for 15, 30, or 45 minutes. Thereafter the supernatant was drawn off and the cells were cultured for 48 hours in petri dishes coated with a 2% agarose gel. Unpanned cell suspensions were cultured for comparison. The cells were then fixed and stained with myosin antibody MF20 (courtesy of Dr. Don Fischman, Cornell Medical School) and propidium iodide (a nuclear stain) to calculate the percentage of myoblasts. This experiment revealed that the greatest myoblast enrichment was obtained after a panning time of 15 - 20 minutes.

Panned cells were centrifuged to form a pellet, which was placed in a sterile dish and dissected into fragments measuring approximately 150 x 250 microns. The fragments were used for either in vitro viability assays or surgical transplantation. The former confirmed that differentiation-competent myo-

blasts survived the preparative procedures.

Using electrolytically-sharpened tungsten needles, a trough was cut into paraxial mesoderm at the midbrain-hindbrain border of a 36-hour (10-somite) chick host embryo, and the pelleted quail myoblasts were grafted into this site. After five days, the surviving chimeras were fixed, sectioned, and Feulgen-stained to visualize the nuclear marker characteristic of quail cells.

Donor quail cells survived the transplantation and differentiated into multinucleated skeletal muscle and loose connective tissues at the site of implantation within the host chick embryo. No evidence of myoblast migration was observed.

These in vitro and in vivo results defined optimal conditions for isolating and enriching for myogenic-competent myoblasts from developing muscle tissues of varying ages. The transplantation studies prove that myogenic cells from limb muscles of older embryos are competent to differentiate in the head region of much younger embryos. In the future, this assay system will be expanded to assess the abilities of myoblasts from young or old embryos to migrate and differentiate along specific pathways (e.g. 1° vs. 2°, slow vs. fast) when challenged by the early embryonic microenvironment.

I greatly appreciated having the opportunity to participate in the Leadership Program for Veterinary Students. I would especially like to thank Drs. Drew Noden and Darrell Evans for their guidance, enthusiasm, and patience during my project. I would also like to thank the Mellon Foundation and the NIH for their support, Dr. McGregor and Linda Griswold for organizing the program, and the many friends I made for making this summer a truly rewarding and enjoyable experience.

## Allison Stewart - University of Melbourne



As a final year student at the University of Melbourne, I am intrigued by the diversity of challenges open to veterinary scientists. To attend the Leadership Program I took a year's leave of absence. This allowed me not only to enroll in the program but also to work in several veterinary facilities in the US, Canada and Europe.

The ten weeks at Cornell was the most valuable summer experience of my life. As I search for my niche within the veterinary profession, my interest has been aroused by the opportunities and rewards of applied research.

Duchenne muscular dystrophy (DMD), is a recessive X-linked condition characterized by dystrophin deficient myocytes, progressive muscle weakness and skeletal muscle necrosis. Succinylcholine is a depolarizing neuromuscular blocking agent, that is used routinely to facilitate tracheal intubation during human anesthesia. When succinylcholine is administered to patients with DMD, it may provoke a syndrome characterized by acute metabolic acidosis, hyperthermia, rhabdomyolysis, myoglobinuria, severe hyperkalemia, tachycardia and ventricular fibrilla-

tion which is often fatal.

My objective was to determine whether golden retriever dogs with a genotypic and phenotypic homologue of DMD, could be used to reliably reproduce the adverse effects of succinylcholine.

I hypothesized that the sarcolemma of dystrophin deficient cells would be sufficiently defective to allow hyperkalemia as a result of massive synchronous myocyte contraction caused by the succinylcholine induced depolarization. I further hypothesized that this hyperkalemia would be sufficient to produce cardiac dysrhythmias.

My study involved five dogs — one normal and four affected animals. Under pentobarbitone anesthesia, the subjects were mechanically ventilated. Instrumentation consisted of a capnograph, esophageal temperature probe, electrocardiogram and electromyogram. A force transducer and ulnar nerve stimulator were used to record the completeness and duration of neuromuscular blockade. The dorsal pedal artery was catheterized for arterial blood pressure monitoring, and for sampling to measure arterial blood gas tensions, electrolytes, ionized calcium, PCV, plasma creatinine kinase (CK) and aspartate transaminase (AST). Cranial tibial muscle biopsies were performed before, and four hours after the succinylcholine challenge of 0.3mg/kg.

The experimental subjects did not exhibit the extreme degrees of muscle rigidity, metabolic acidosis or malignant hyperpyrexia reported as a sporadic occurrence in children with DMD. It was therefore concluded that the canine homologue is not a reliable model for this phenomena. However, there was an unexpected delayed hyperkalemia in all dogs, with CK rebound in four of the five dogs, 24 hours after treatment with succinylcholine. Three dogs showed a modest immediate

CK increase after five minutes, while potassium levels rose in all cases. Succinylcholine thus altered membrane permeability in both normal and affected dogs.

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**It was concluded that dogs with Duchenne muscular dystrophy do not respond to succinylcholine with the same drastic consequences as affected humans.**

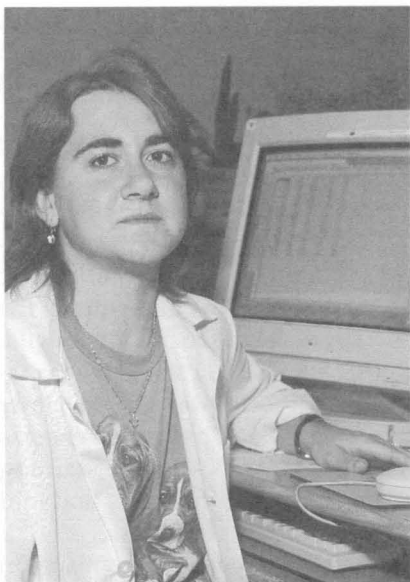
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Frozen sections were cut from biopsies and stained with H&E and trichrome stains. Muscle necrosis, regeneration and large dark fibers characteristic of muscular dystrophy were seen. No histopathologic differences between pre and post succinylcholine biopsies were found.

This summer, I worked with Barry Cooper and Robin Glead, whom I would like to thank for the guidance and wisdom they were so willing to share. In addition I am extremely grateful to Tasha, Teena and Yelena for their patience and assistance. I greatly appreciate the support of the Merck Foundation, as well as the enthusiasm and dedication shown by Linda Griswold and Dr. McGregor. The insights and knowledge I have gained, and the friendships I have formed will certainly be with me forever.



## Justine Swaney - University of Tennessee



This summer I worked with Dr. Hussni Mohammed in the Section of Epidemiology, Department of Clinical Sciences, on the epidemiology of equine protozoal myeloencephalitis (EPM), a neurological disease caused by the organism *Sarcocystis neurona*. The disease is marked by a number of clinical signs such as ataxia and paresis, and can mimic other neurological conditions, making an antemortem diagnosis difficult. Immunoanalysis of suspect serum or cerebrospinal fluid (CSF) for antibodies to *S. neurona* offers a more specialized diagnostic aid; however, the accuracy of the tests in identifying a diseased horse is questionable.

The purpose of my study was two-fold. First, I investigated the accuracy of serum and CSF immunoanalysis as antemortem diagnostic tests, using a histopathological diagnosis of EPM as a gold standard. Secondly, I examined potential risk factors associated with the disease and quantified their risk.

For the diagnostic test analysis, samples for immunoanalysis obtained by the Cornell University Veterinary Hospital from September, 1992 to July, 1996 were consid-

ered. Testing was performed by the University of Kentucky Equine Biondiagnostics, Inc. Results were recorded as "positive" or "negative" for antibodies to *S. neurona*. The accuracy of the tests was measured in terms of sensitivity and specificity. Sensitivity was defined as the probability that a horse with EPM (as confirmed by histopathology) would test positive for antibodies to *S. neurona* in serum or CSF. Specificity was defined as the probability that a horse without EPM would test negative for such antibodies.

For the risk factor analysis, we used a retrospective case-control study of histopathologically or immunoanalytically diagnosed EPM cases from 1990 to 1996 (58 cases). One hundred control subjects were selected from horses confirmed as having cervical stenotic myelopathy, equine degenerative myelopathy, or equine motor neuron disease. These neurological diseases can present similar clinical signs as EPM. Information on four putative risk factors (age, sex, breed, and month of diagnosis) was collected for all cases and controls. Multivariate logistic regression analysis was used to evaluate potential associations between these risk factors and the likelihood of EPM.

As an antemortem diagnostic test, serum immunoanalysis was found to be highly sensitive in detecting antibodies to *S. neurona*. Specificity was quite low, however, as prior exposure to the organism can also cause a detectable antibody level in the serum, indistinguishable from that seen in an active disease state. The predictive value of a positive serum test in indicating disease is thus diminished, and there are a high number of false positives. CSF immunoanalysis, although somewhat more specific, still did not meet either the 90% sensitivity or specificity claimed by a previous

report. In the risk factor analysis, there was a breed and age association with the risk of EPM. Standardbreds were at a high risk for developing EPM, with an odds ratio of 25.2. Thoroughbreds were also at an increased risk, with an odds ratio of 4.0. The age association was nonlinear, with the risk of EPM reaching a peak at around eight years of age. No seasonal or sex association was detected.

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### As an antemortem diagnostic test, serum immunoanalysis was found to be highly sensitive in detecting antibodies to *S. neurona*.

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I would like to thank the NIH and Merck Foundation for sponsoring my research this summer and Maura Westerdahl for her help in getting my project off the ground. Special thanks goes to Dr. Mohammed, who showed me that there is more to epidemiology than Ebola outbreaks, and who gave me the opportunity and independence to pursue a project that I could see through from beginning to end — from literature review to publication. He also puts up a fair fight in tennis. Finally (and hopefully without getting too cheesy) I would like to thank the other Leadership students for making this an exceptionally fun summer, a "shocker," if you will. I really feel that we created a home away from home at 660 Stewart, where we grew to know each other, be comfortable with each other ... and beat each other in a friendly game of pool.

## Alicia Uixera - University of Zaragoza



Veterinary Medicine has always been my passion. Last September I finished my fifth year at the University of Zaragoza (Spain). During my clinical training over the last two years, I have discovered my inclination for ruminants and small animals. The leadership program gave me the opportunity to approach the research world.

This summer I worked in the Reproductive Pathology Research Laboratory with Dr. Don Schlafer. We endeavored to identify cells in the bovine placenta that may be associated with inflammation and immunity, and then to assess their role in acquired resistance to transplacental infection. The focus of my project was the identification of macrophages in fetal bovine tissues.

Macrophages have a central role in placental defense but, paradoxically, they may also be involved in the transmission of some infectious diseases, such as AIDS, across the placenta to the developing fetus. Although macrophages are an important cellular constituent of the placenta, little information is available about their number and functions in the placentas of domestic animals.

Antibodies against human mac-

rophages that cross-react with macrophages of several other species may provide important tools for exploring these issues. Three commercial antibodies were screened for use in bovine tissues: HAM56 (Enzo), CD68(EMB11)(Dako), MAC387(Dako). Cz1 was used as a negative control.

Sections of normal bovine lymph served as the "positive control tissues," while tissues from three spontaneous cases of bovine candida placentitis served as placental controls for fetal macrophages. The histochemical technique employed was a biotin-streptavidin complex immunoperoxidase system using formalin-fixed, paraffin-embedded fetal and maternal tissues. Initially, different staining conditions and antibody dilutions were tested on control tissues to define the optimum staining conditions for macrophages.

I found that optimal staining was achieved for CD68(EMB11) (1/30 dilution) using trypsin and 2 h primary antibody incubation at 37°C, while HAM56(1/100) required microwaving and overnight primary antibody incubation at 4°C. We did not use MAC387 because of its cross reaction with neutrophils.

Selected maternal tissues and 20 specific fetal and placental tissues were collected from each of 9 pregnant cows from a slaughterhouse. Three fetal age groups were sampled: early(100-120 d.gest), mid(190-200 d.gest), and term (>240 d.gest). Evaluation of two complete sets of tissues from the first two groups and three from the term group (total of 140 tissues) enabled us to draw the following conclusions:

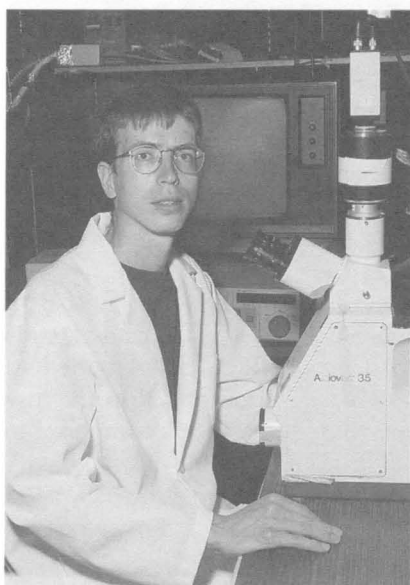
1. CD68(EMB11) is an excellent antibody for staining bovine macrophages in fetal and maternal tissues. It labels macrophages in fetal spleen, liver, bone marrow, gut and choroid

plexus. Maternal tissues (uterus, caruncular septae and endometrium) also contained many brightly labeled macrophages. Within the placental chorioallantois, macrophages were present within the chorionic epithelium, in the perivascular areas of the allantoic stroma and in aggregates in the stroma of the cotyledonary villi.

2. CD68(EMB11) stains macrophages more intensely in "later pregnancy" placenta whereas HAM56 gives better staining when applied to early and mid pregnancy placentas.
3. HAM56 labels macrophages in placentas but, surprisingly, and in contrast to CD68(EMB11), it does not stain macrophages in normal uterine tissues. We are unaware of any published reports on the use of HAM56 to label macrophages in bovine tissues, suggesting that this antibody will be a useful tool for future use.
4. We also detected mature macrophages in fetal tissues even in young fetuses (100-120 d.gestation). Using the two antibodies in serial tissue sections, different staining patterns were observed. The differences were interpreted as suggesting that the degree of macrophage activation varied throughout gestation.

I would like to thank my mentor for allowing me to "sail into the research" world with relative freedom (even if some times I felt a bit lost). Thanks also to the people who have been working hard to make this program possible and to my sponsor Fundación Purina for giving me this opportunity.

## Edwin van Duijnhoven - University of Utrecht



Growing up on my parent's dairy farm and working with their cattle have been my main reasons for studying veterinary medicine. I interrupted my veterinary training for one year to explore research as a career option, and I received the degree of Master of Veterinary Research. I am now entering my final year of the veterinary program at the University of Utrecht.

I felt privileged when I was selected for the Leadership Program this summer knowing that it would afford me an opportunity to further explore career options. I also had the pleasure of working with Dr. Susan Suarez in the Department of Anatomy. My project focused on characterizing sperm binding to oviductal epithelium.

In large mammals, millions of sperm are inseminated to fertilize one, or at most, a few oocytes. Only a few thousand reach the isthmus of the oviduct, however. There, most of them are held back in a reservoir. The oviductal reservoir for sperm may serve a number of functions: prevention of polyspermy by allowing only a few sperm to reach the oocyte at a time; maintenance of the fertility of sperm between the onset of estrus and fertilization, and

regulation of the process of capacitation of the sperm. (Capacitation is a set of changes in the sperm plasma membrane that enables sperm to undergo acrosome reactions and fertilize oocytes.)

### My project focused on characterizing sperm binding to oviductal epithelium.

A graduate student from Dr. Suarez's laboratory, Dr. Rejean Lefebvre, demonstrated that bovine sperm binding to oviductal epithelium involves fucose and that there is a fucose-containing ligand on the oviductal epithelium. My task was to look for the fucose-lectin molecule on bull sperm. Furthermore, we wanted to test the hypothesis that this lectin is lost or modified during capacitation. To localize the lectin on sperm, we first used a fluorescein isothiocyanate labeled, fucosylated bovine serum albumin molecule. We expected labeling over the acrosomal region, because bull sperm bind mostly by their heads to oviductal cells. As it happened, we detected only faint labeling over the post-acrosomal region.

Despite using several protocols, we were not able to enhance the intensity of the label and attempting to block that labeling with fucose gave no reward. Therefore it seems likely that the observed fluorescence was non-specific.

In the next series of experiments, we used fucose-agarose beads to explore sperm-fucose interactions. Linkage of sugars is often a determinant of binding specificity, so we chose these beads because there was a different linkage than there was on fucosylated albumin. The beads consist of fucose linked to a poly-galactose gel. To

examine that interaction with sperm, we used differential interference contrast optics. Unfortunately, these experiments also did not provide evidence for a fucose-lectin on bull sperm, for we could not demonstrate sperm binding to the beads.

Next, we tried a molecule with still another linkage: fucoidan. Fucoidan is a polysaccharide composed predominantly of sulfated fucose which can agglutinate sperm. We did succeed in demonstrating this, but were unsuccessful in blocking the agglutination with fucose. The affinity of the sperm for fucoidan is perhaps much higher than for fucose.

To test the latter hypothesis, we incubated capacitated sperm with fucoidan. Agglutination was observed to the same degree as uncapacitated sperm. Hence, our second hypothesis could not be supported either.

This research program has been an even greater experience than I had expected. It enabled me to learn more about alternative career paths for veterinarians, and gave me a good impression of basic research. Also, the social aspects and events outside the formal program and laboratory broadened my mind so I will return to the Netherlands feeling very enriched. Therefore I would like to thank the Merck Foundation for offering me a fellowship; Dr. Susan Suarez for being an excellent mentor; the program organizers, and everyone else who contributed to it.



## Ilse van Vonderen - University of Utrecht



As long as I can remember I have wanted to become a veterinarian. In 1990 I enrolled at the University of Utrecht, Faculty of Veterinary Medicine, and this fall I will enter my final clinical year. Although I started studying veterinary medicine with the sole intention of going into private practice, my views changed when I took a year off in 1994 to perform clinical research. I spent 1994 at the Department of Clinical Sciences of Companion Animals studying the mechanisms of polyuria/polydipsia in dogs. This was my first exposure to research and I thoroughly enjoyed the experience. Suddenly my horizons were broadened, and I realized how many more possibilities there are for veterinarians and especially how challenging and exciting research can be. I applied to the Leadership Program as seeing in it an excellent opportunity to learn more about research careers for veterinarians, and to meet like-minded colleagues from all over the world. I was thrilled to be accepted.

This summer I worked with Dr. Tom McDonald in the Laboratory for Pregnancy and Newborn Research studying the effect of glucocorticoids on the expression of glu-

cocorticoid receptors (GR) in CRH cells of the sheep hypothalamus. Glucocorticoid secretion is regulated by hormonal interactions between the hypothalamus, pituitary and adrenals (HPA) and by neural and stress stimuli. The feedback control of the HPA-axis is mediated by glucocorticoid receptors. According to classical feedback regulation, glucocorticoids can inhibit not only the production of hormones like CRH and ACTH, but also the production of their own receptor. Studies in rats have demonstrated the occurrence of GR down-regulation by increased levels of glucocorticoids in the hippocampus but not in the hypothalamus. The use of whole hypothalami in binding assays, however, may not reflect significant changes in the relatively small number of CRH cells.

### **I studied the effect of glucocorticoids on the expression of glucocorticoid receptors in CRH cells of the sheep hypothalamus.**

We hypothesized that by using immunocytochemistry we could demonstrate that glucocorticoid administration results in a dose-dependent down-regulation of GR in CRH cells of the sheep paraventricular nucleus. For this experiment 15 adult Rambouillet-Columbia ewes were divided into three groups. After progesterone sponging, ovariectomy and five days of recovery, the animals received a 48-hour infusion with either saline (control group) or 0.75 or 2.5 µg/kg/h of the glucocorticoid betamethasone (test groups). We also collected morning and afternoon blood samples for determination of ACTH and cortisol levels. At the end of the infusion the animals

were euthanized and their brains were collected. The hypothalamus was sectioned at 20 µm using a sliding microtome, and double staining studies for GR and CRH were performed using the avidin-biotin complex method. In this way it is possible to count the number of CRH cells that have a GR positive or negative nucleus. After statistical analysis of the data from all animals we expect to find a dose-dependent down-regulation of GR in the low and high dose groups compared to the saline control, resulting in a decrease in the percentage of CRH cells that have a GR positive nucleus.

I would like to express my gratitude to my mentor, Dr. Tom McDonald, for his infinite patience in answering my questions, for his encouragement when experiments failed, and for sharing with me all the fun and frustration that go hand-in-hand with research. I would like to thank Dr. Cun Li for teaching me the laboratory techniques and Dr. Xiu-Ying Ding for giving me the opportunity to acquire surgical experience. I am furthermore grateful to Dr. Peter Nathanielsz, Lynn Buchwalder, and all the other personnel and students at the LPNR for making this summer such an enjoyable and worthwhile learning experience.

My participation in the Leadership Program would not have been possible without the support of my sponsor, the Simpson Trust, and Dr. McGregor, Linda Griswold and Allan Kaplan as organizers of the program. But my special thanks go to the 23 other students in the program with whom I experienced a summer we all shall never forget.

## Constantin Von der Heyden - University of Pretoria



Currently I am a fourth year student at Onderstepoort, the Faculty of Veterinary Science of the University of Pretoria. My aim has always been to follow a career in academia, and especially research. Therefore I was very fortunate to be offered a Merck fellowship position in the 1996 Leadership Program. Because of my interest in pharmacology and the central nervous system (CNS), Dr. Linda Nowak of the Department of Pharmacology has acted as my mentor. Her specialty is the study of physiological mechanisms of mammalian CNS neurotransmitter function, with glutamate and its receptors as her primary focus.

Glutamate is a ubiquitous amino acid that acts throughout the CNS as an excitatory neurotransmitter. Many of its physiological effects are counteracted by the inhibitory neurotransmitter gamma amino butyric acid (GABA). In fact, it is when this fine balance of glutamatergic excitation versus GABAergic inhibition is disrupted that signs of disease manifest, such as seizure activity, neural hypoxia and ischaemic necrosis leading to neuronal death. Glutamate is also associated with the higher functions

of learning and long-term memory.

There are principally two groups of glutamate receptors, the metabotropic and the ionotropic. Metabotropic receptors are G-protein coupled, and the binding of glutamate leads to the activation of intracellular signaling cascades. On the other hand, the ionotropic receptors are pores which form cation channels when bound by glutamate. Two main subgroups have been identified, namely the NMDA (N-methyl-D-aspartate) and the non-NMDA channels. The NMDA channels are highly calcium permeable, with a calcium to sodium ( $P_{Ca}/P_{Na}$ ) permeability ratio of between 7:1 and 10:1. Non-NMDA channels are again subdivided into AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid) and kainate receptors, named after their respective agonist molecules.

Typically, AMPA receptors are less significantly calcium permeable ( $P_{Ca}/P_{Na}$  ratio of 0.5:1). Dr. Nowak's lab is working with a particular class of AMPA receptor found in 14.5 day old mice embryos, which is not commonly found in the adult mouse. This receptor has a  $P_{Ca}/P_{Na}$  ratio of 3:1 and is speculated to be important in neuronal development and the differentiation of progenitor cells. Of the 7 cDNA (GluR1-GluR7) sequences that code for the non-NMDA protein subunits, GluR1, GluR3 and GluR4 are likely candidates to form this particular receptor.

My project started with cutting GluR1 and GluR3 from their bacterial plasmid vector, pBlueScript (pBS), using restriction enzymes. Each of these subunits was then ligated into two different mammalian expression vectors, pRK5 and pUHD10-3. Restriction digests were run to show diagnostic bands on electrophoresis, so verifying our progress. An *E.coli* strain (DH5 al-

pha) was transformed with the above mentioned plasmids, as well as the green fluorescing protein (gfp) plasmid and transformed cells were selected. Plasmid DNAs were extracted from maxipreps and purified by CsCl gradients. Human embryonic kidney (HEK 293) cells, passaged onto collagen coated coverslips, were transiently transfected with the pRK5/GluR1 as well as the gfp plasmid, using Lipofectin and Lipofectamine. Ratios of 10:1 and 5:1 of pRK5/GluR1 and gfp respectively, were used in transfections. In this way the probability was substantially increased that the cells that showed fluorescence indeed contained the required GluR1 sequence. Preliminary results suggest the Lipofectamine protocol will work better for transient transfections. I also began the procedure of stably transfecting cell lines. This time a plasmid that coded for neomycin resistance was co-transfected into the cells along with pRK5/GluR1. Cells that did not take up the neomycin plasmid were killed when geneticin was introduced into the medium 3 days following the procedure. Electrophysiological studies will be carried out to compare characteristics of the AMPA channels expressed on these cells to non NMDA channels obtained from the 14.5 day old embryonic neurons.

I have learned much during my short stay in the United States and would like to thank Linda Nowak and Mary Robison for their endless patience, help and support. My appreciation also goes to Dr. McGregor for initiating and coordinating a program in which students from around the world can come together and experience what we have this summer.



*Dr. Janice Bicknese presenting Dean Franklin Loew with a check representing the Merck Foundation's contribution to the Leadership Program.*



*Edwin van Duijnhoven and Dr. Barry Cooper at the Leadership dinner.*



*Musicians entertained guests attending a dinner following the leadership discussion.*



## Acknowledgments

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### NOTES:

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