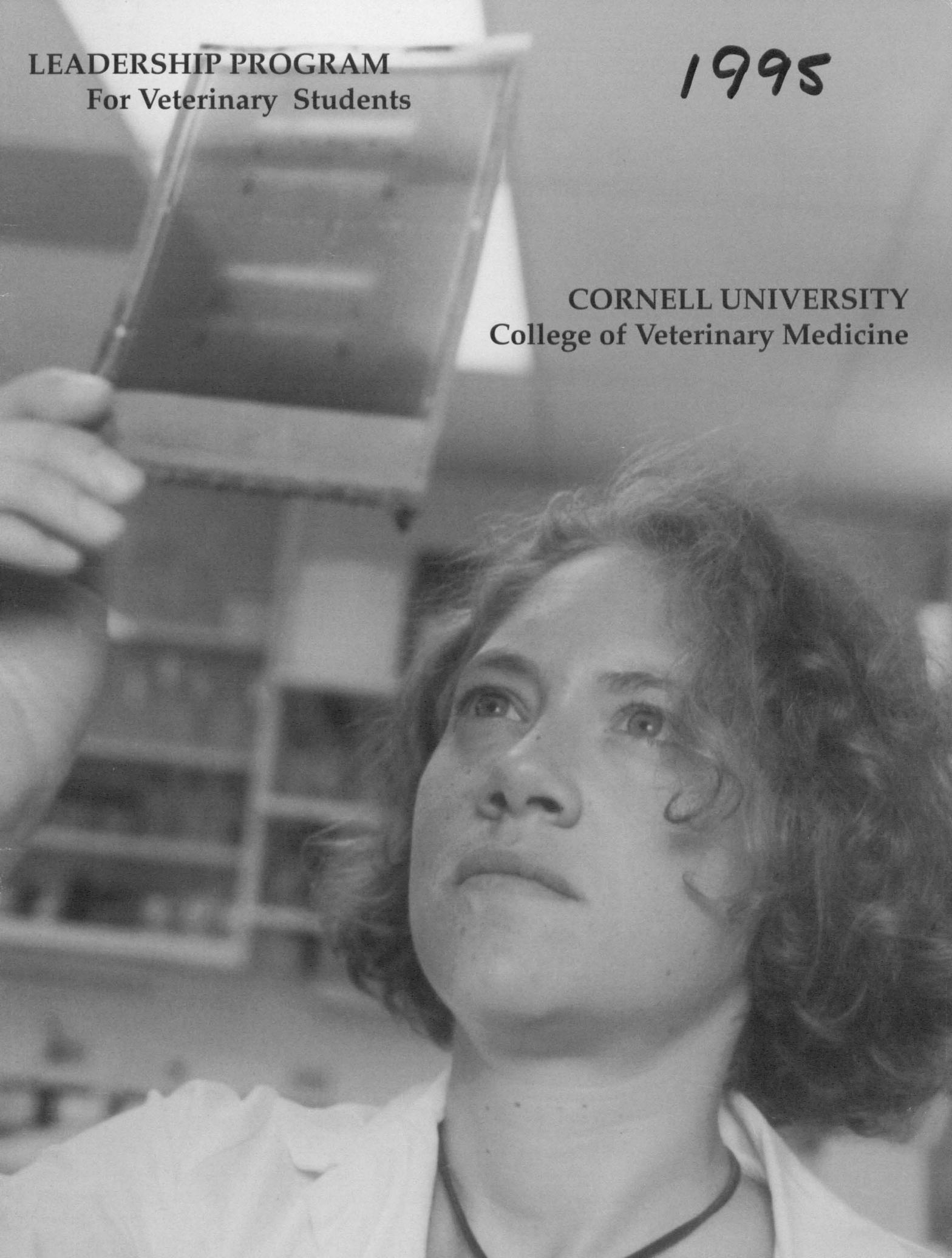


LEADERSHIP PROGRAM
For Veterinary Students

1995

CORNELL UNIVERSITY
College of Veterinary Medicine



Program Overview

The College of Veterinary Medicine at Cornell University hosted the sixth annual Leadership Program for Veterinary Students this year. The program targets students who have leadership potential and have achieved distinction both academically and in a variety of personal pursuits. It is anticipated that many program "graduates" will realize their career ambitions in an academic setting or as research scientists in government or industry.

Twenty-four students from 14 different veterinary colleges in the United States, Canada, Australia, New Zealand, the United Kingdom, Germany, and Spain took part in this year's program. Joshua Weisberg, a Cornell undergraduate and aspiring veterinary student, served as "Program Coordinator." Elizabeth Barnes (University of Illinois) and Krista-Britt Halling (Ontario Veterinary College) were elected by their fellow students as "Program Representatives." Both had a major role in facilitating events connected with the program.



"Class of 1995"

The program spanned ten weeks during the months of June, July and August. It was an active learning experience in which the student fellows assumed major responsibility for organizing modules and working collegially with an outstanding group of faculty members, discussion leaders, and consultants.

Fellows were assigned 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. They also had individual learning assignments and took part in several group projects. The latter focused on 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 the United States Department of Agriculture were additional features of the program.

Career Counseling: 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 in an advanced stage in their own training, served as discussion leaders. Taking part this year were:

- Dr. Prema Arasu, Assistant Professor of Pathobiology, 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. Alastair J.S. Summerlee, Vice President, Programs and Production, Lifelearn, Inc., University of Guelph



Dr. Linda Rhodes discussing career opportunities for veterinarians.

B. Carter, Professor of Microbiology at North Carolina State University, and the Leadership Program Director, Dr. Douglas D. McGregor, served as discussion leaders. The students identified the characteristics of a leader, attached priorities to these attributes, then shared their conclusions with members of the other two groups.

Biomedical Ethics: Reflection on ethical issues connected with the conduct of research and veterinary practice is one of several themes in the Leadership Program. Two modules addressed these issues this year. Both were organized by Professor Ari van Tienhoven, who has taken the lead in this aspect of the program for several years. Students had the option of participating in either module. One was organized as a case study in which the students considered ethical issues connected with the ownership and publication of data, data fabrication, and power relationships. The second module considered the influence of Darwinian evolution on the foundation of biomedical ethics. The discussion was led by Professor William B. Provine, a distinguished evolutionary biologist who engaged the students individually in dialogues that considered the topic in several different contexts. Two other Cornell faculty members, Professor van Tienhoven and Professor Fred W. Quimby, joined the student panel as did Dr. Strachan Donnelley, Director of Education at the Hastings Center. Aside from its intrinsic interest and relevance to biomedical ethics, the module afforded the opportunity for students to exercise their critical thinking skills.

The students and counselors developed an agenda during the evening prior to the meeting. The meeting itself featured presentations by the counselors and breakout sessions in which students and counselors explored aspects of career planning. Later that evening the students entertained their visitors and other invited guests at a formal dinner.

Professors Robert M. Lewis and N. Sydney Moise led an evening discussion of internship and residency training. The discussion centered on factors that veterinary students should weigh in evaluating clinical and pathology training programs. At a separate meeting, Professors Barbara A. Baird, Richard A. Cerione, and Edward J. Pearce discussed similar issues connected with graduate research training. On still another occasion, two scientists from the research laboratories of Merck & Company, Dr. Gerard J. Hickey and Dr. Michelle L. Haven, visited the College to discuss career opportunities for veterinarians in industry. They described their own research, administrative duties, and commented on differences between research conducted in an academic institution as opposed to a research intensive pharmaceutical company.

Many of the events connected with the program enabled students to assume responsibility and to reflect on the personal and professional aspects of leadership. On one occasion, the students discussed these issues in a more formal way. To this end, they divided themselves into three groups. Dr. Norman R. Scott, Vice President for Research and Advanced Training at Cornell, Dr. Philip



Rachael Gray, Ross Koenig, and Marcus Gunew discussing the characteristics and responsibilities of leadership.

Radiation Safety: Radioisotopes are used frequently in research, yet many inexperienced investigators are unfamiliar with procedures connected with their proper acquisition, use and disposal. Participants in the 1995 program received four hours of instruction on these matters. Presentations were arranged by Cornell's Radiation Safety Officer, Mr. Thomas J. McGiff.

Life Safety: Dr. Larry J. Thompson, Director of Biosafety in the Veterinary College, presided at a meeting that reviewed principles governing the handling, storage and disposal of hazardous chemicals. Dr. Thompson also reviewed the College's policy regarding the protection of employees from workplace hazards, including exposure to rabies virus.



Professor and Mrs. William Provine being greeted by Danielle Greenberg.

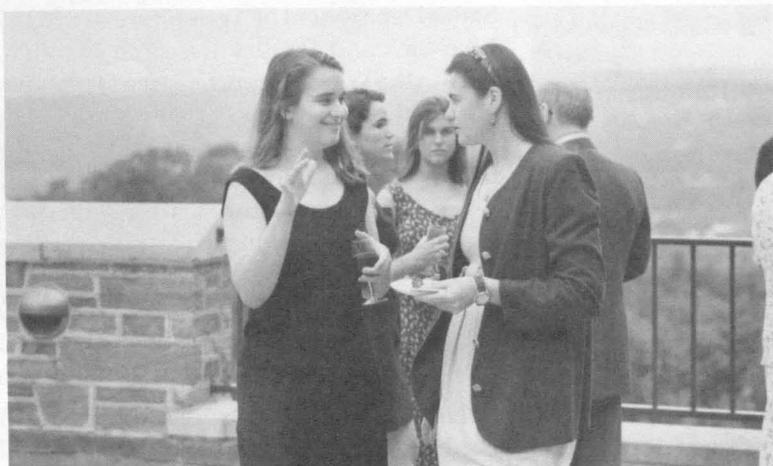
Site Visits: The students and several members of the participating faculty visited the research facilities of the National Institutes of Health (NIH) and the United States Department of Agriculture's (USDA) Livestock and Poultry Research Institute, both in the Washington, DC area. The visits afforded opportunities for the students to meet with senior scientists and administrators, and to gain insight into the scope of research and opportunities for advanced training in the host institutions. Agendae for the visits are reproduced below.

**National Institutes of Health
June 22, 1995
Research and Research Training at the NIH**

8:45 a.m.	Ms. Maureen Mylander, NCRR Public Affairs Officer, "Welcome, NCRR Video"
9:00 a.m.	Ms. Pamela Dressell, Chief, Client Relations, VRP, "Overview of NIH, NCRR and NIH Veterinarians" Dr. Melvin Dennis, Director, VRP, "The Role of Veterinarians in Laboratory Animal Programs" Dr. Victoria Hampshire, Chief, Carnivore and Ungulate Section, Research Animal Branch, VRP, "Transgenic Swine Project" Dr. Michael Eckhaus, Chief, Pathology Unit, Laboratory Sciences, VRP, "Pathogenicity of Carbacillus in Immunodeficient Mice"
10:30 a.m.	Dr. Clarence Gibbs, Deputy Chief, Laboratory of Central Nervous System Studies, "Transmissible Spongiform Encephalopathy"
11:30 a.m.	Mr. James Alexander, NIH Office of Education, "NIH Research Fellowship and Training Opportunities"
12:30 p.m.	LUNCH
2:00 p.m.	Ms. Martha Kirby, M.S., Research Biologist, NHLBI Clinical Hematology Branch, "Primate Gene Transfer and Bone Marrow Transplantation Program" Tour of Facilities
4:45 p.m.	Final Review

**United States Department of Agriculture
(Beltsville Campus)
June 23, 1995**

8:30 a.m.	Dr. Gordon Marten, Associate Area Director, "Overview of the Beltsville Agricultural Research Center"
8:45 a.m.	Dr. T.J. Sexton, Institute Director, "Overview of the Livestock and Poultry Sciences Institute"
9:00 a.m.	Dr. Max Paape, Research Dairy Scientist, Milk Secretion and Mastitis Laboratory, "Mastitis Research"
9:30 a.m.	Dr. Ron Fayer, Zoologist, Parasite Immunobiology Laboratory, "Cryptosporidiosis"
10:00 a.m.	Break
10:30 a.m.	Dr. Robert Wall, Research Physiologist, Gene Evaluation and Mapping Laboratory, "Transgenic Animal Research"



Christine Harshbarger and Kellie Stephenson at a reception preceding the Leadership Day dinner.

Student Presentations: At the conclusion of the program, the student fellows reported on their research activities. The presentations were uniformly good, and some were outstanding. The following individuals were awarded book prizes:

- Derek Wilson - Program Prize
- Adrienne Bentley - Molecular Biology Prize
- Krista-Britt Halling - Cell Biology Prize
- Wendy Harrison - Veterinary Medicine Prize



Dr. Patricia Chisholm, Director of the Veterinary Programme at The Wellcome Trust and three Wellcome-sponsored fellows, Caroline Murray, Danielle Greenberg, and Wendy Harrison.

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

Eileen Adamo - Cornell University



After receiving my Bachelor of Science in Biochemistry from Cornell University in 1993, I enrolled in the Cornell University College of Veterinary Medicine, Class of 1997. I have been interested in research since my undergraduate years. In fact, I entered veterinary school with the intent of gaining a clinical foundation on which to build my research career. The Leadership Program has been helpful in that regard insofar as it exposed me to the diverse research opportunities available to veterinarians.

My project this summer was conducted in Dr. Bendicht U. Pauli's laboratory in the Department of Pathology, Section of Cancer Biology. Dr. Pauli's team is studying tumor cell/endothelial cell interactions during vascular arrest and extravasation of blood-borne cancer cells. A lung-derived endothelial cell adhesion molecule, Lu-ECAM-1, has recently been isolated, purified, and cloned. Lu-ECAM-1 mediates lung vascular arrest and metastasis of lung-metastatic B16-F10 melanoma cells. It appears to regulate the retraction of endothelial lining cells, allowing passage of the tumor cells out of the vasculature and into the lung parenchyma

where they establish a metastatic tumor mass.

The specific goal of my project was to use various molecular biological and cell culture techniques to determine whether a soluble form of Lu-ECAM-1 is secreted by endothelial cells. To this end my energy was devoted to two major experiments. First, I sequenced a cDNA clone of Lu-ECAM-1 called 25-1 that was thought to encode a secreted protein product (based on restriction fragment analysis). I found that the 25-1 clone was truncated within the N-terminal extracellular domain of Lu-ECAM-1, encoding a protein of 320 amino acids that lacks a transmembrane region and is therefore probably secreted.

Next, I looked for the predicted protein in "conditioned medium" harvested from cultures of bovine aortic endothelial cells (BAECs). I recovered the frozen stock cells and grew them to confluency on culture plates. I then transferred the cells to serum-free medium and collected this conditioned medium at intervals of one, three, and seven days. Thereafter, I concentrated the media 200-fold, and ran the sample on acrylamide gel, then transferred the proteins to Immobilon nylon for a Western blot. Three signals at 60kD, 45kD, and 33kD were seen in lanes incubated with affinity-purified primary antibodies, but not in the lane incubated with preimmune primary antibodies. Either the 45kD or the 33kD protein could correspond in size to that predicted by the 25-1 cDNA clone; however, the 60kD band was too large to be explained by this cDNA.

Next, conditioned medium was immunoprecipitated with anti-Lu-ECAM-1 monoclonal antibody 6D3. The precipitates were subjected to western analysis as described above. We were again pleased to observe a 45kD band. We also still observed some bands in the higher molecular

weight range which we decided were probably aggregates of the 45kD protein.

In summary, we have established that soluble extracellular forms of Lu-ECAM-1 do exist, although their biological activity has yet to be determined. It is possible that soluble Lu-ECAM-1 could have a protective role in inhibiting binding of tumor cells to vascular endothelium by simple competitive inhibition for receptor sites. However that may be, it could be of value as a prognostic indicator of tumor progression since the concentration of Lu-ECAM-1 would be expected to increase as more Lu-ECAM-1 expressing endothelial cells are recruited during tumor neovascularization to support the expanding tumor mass.

...Lu-ECAM-1 could have a protective role in inhibiting binding of tumor cells to vascular endothelium...

I am greatly indebted to the NIH and the Robert W. Woodruff Foundation which funded my research this summer. I also wish to thank Linda Griswold and Joshua Weisberg for their administrative efforts, and Dr. McGregor for his commitment to the program. Finally, I especially would like to thank Dr. Pauli, Dr. Elble, and everyone in the lab who supported me with their patience, enthusiasm, and expertise. I learned a great deal this summer and have had an excellent research experience through the Leadership Program.

Elizabeth Adkins - University of Tennessee



I want a broad range of experiences from my veterinary education and feel fortunate to have been selected as a participant in the 1995 Leadership Program. Before coming to Cornell, I had several years of clinical experience. During the summer of 1993, I was engaged in a behavioral study under the direction of Dr. Christine Boake at the University of Tennessee. A paper produced from this study was published in the *Journal of Insect Behavior*.

This summer I worked in the Levine Laboratory with Dr. K.A. Schat. I initiated the cloning and sequencing of the genome of Duck Virus Enteritis (DVE). DVE is an acute, contagious herpesvirus infection of domestic and wild ducks, geese and swans. DVE is transmitted by direct contact with infected birds or by contact with a contaminated environment. Wild waterfowl can be protected from this disease by preventing contamination of their habitat.

A chicken embryo adapted DVE strain is available as a vaccine for domestic birds. Outbreaks of this disease in wild waterfowl inspired the current interest in alternative vaccines.

The clinical signs of DVE include: a sudden, high and persistent mortality rate within the flock; inappetance, ataxia, soiled vents and watery diarrhea. The incubation period is three to seven days and the birds usually die one to five days after the onset of clinical signs. The gross lesions are pathognomonic; they include: necrosis of the gastrointestinal epithelium, liver, lymphoid tissue and blood vessels. Intranuclear inclusion bodies are seen histologically in gastrointestinal epithelium, hepatocytes and lymphoreticular cells.

I initiated the cloning and sequencing of the DVE genome to allow characterization of the DNA and possible detection of vaccine candidates. The Cornell University

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Duck Research Laboratory on Long Island provided DVE vaccine strain isolates to be cloned. The plasmid pBluescript was chosen as a vector because it contains a multiple cloning site within the lacZ gene. The cloning site is an area within the plasmid DNA in which foreign DNA can be inserted without interfering with the plasmid's ability to replicate. On selective media, *Escherichia coli* colonies containing the plasmid pBluescript are blue, while colonies that contain a recombinant plasmid are white, because the reading of the lacZ gene has been disrupted by the foreign DNA. The genome of DVE consists of double-stranded DNA. It is estimated to be 150 kbp long. Since it is too large to be cloned as a single piece, I cut it into smaller fragments using the restriction enzyme Bam-HI. The enzyme cuts double-stranded DNA at specific basepair sequences. The vector, pBluescript, was prepared for

cloning by digestion with Bam-HI to linearize the plasmid and insert the foreign DNA. The enzyme Bam-HI recognizes a specific 6bp sequence. Digestion of the DVE DNA and the vector results in homologous sticky ends that will recombine in a ligation reaction. The vector was dephosphorylated to reduce the recombination of pBluescript with itself rather than with the DVE fragments. A ligation reaction of the DVE DNA fragments and pBluescript resulted in recombinant plasmids that were introduced into *E. coli* for replication and amplification.

Eight clones were screened by restriction enzyme digestion and agarose gel electrophoresis. Southern blot hybridization was used to estimate the sizes of the clones by probing DVE DNA with the cloned fragments. Five clones were sequenced and compared to known DNA sequences at the National Institutes of Health computer database. Although substantial progress was made during the ten week period that the program was in session, I had insufficient time to complete the computer analysis of the clones.

I want to thank Dr. Schat for his infinite patience, Priscilla O'Connell for outstanding technical support and Dr. Samia Shawky for providing the DVE isolates. I also want to thank the NIH and the Merck Foundation for sponsoring my fellowship. Special thanks go to Linda Griswold for her assistance prior to my arrival in Ithaca as well as during the program.

Gertraut Altreuther - Free University of Berlin



I am in my final year of vet school at the Free University of Berlin and will receive the veterinary degree early next year. Although I always considered the veterinary education and profession as very attractive, it was not the only idea on my mind when I had to decide what I wanted to do. I love foreign languages and I'm sure that I would have enjoyed becoming a teacher. For a long time I also wanted to be a goldsmith.

I did not have much of a "vet background" when I enrolled in the University of Berlin, having had History and French as majors and having no large animal experience at all. Again it is time for me to decide what I want to do next. Thanks to the Leadership Program I am seriously considering starting off a career in research with a doctoral thesis in molecular biology/pharmacology after my graduation.

This summer I worked with Dr. Kunal Ray at the Center for Canine Genetics and Reproduction at the James A. Baker Institute. One of the major goals of the Center is to identify and study gene defects associated with hereditary retinal degeneration in different breeds of dogs. My summer project dealt with a

retinal disease in Miniature Schnauzers called "Photoreceptor dysplasia."

Photoreceptor dysplasia (*pd*) is a subgroup of the autosomal recessive disease Progressive Retinal Atrophy (PRA). Miniature Schnauzers affected with *pd* go blind within a few years due to slow degeneration of photoreceptor cells. The gene defect causing *pd* is not yet known. During my summer at Cornell, I investigated one of the candidate genes for this disease.

Since *pd* is associated with abnormal photoreceptor cells, it is reasonable to examine genes which are expressed in those cells including genes involved in the process of phototransduction. The latter has a central role in the visual system. I examined the RDS/peripherin gene, which encodes a structural protein of outer disk membranes. The pathology of *pd* is characterized by a structural abnormality of the rod outer segment of photoreceptor cells. The RDS/peripherin is thought to be critical for stabilization of the outer segment. Also defects in the gene causes retinal degeneration in mice and multiple forms of retinal degeneration in humans.

My strategy was to first identify a restriction fragment length polymorphism (RFLP) in the RDS/peripherin gene and then determine whether the RFLP co-segregates with the disease locus. Pursuant to this objective, I amplified a 3.5 kb fragment of the RDS/peripherin gene from genomic DNA by polymerase chain reaction (PCR) using two sets of informative dogs of a *pd*-affected Miniature Schnauzer pedigree. The PCR products were then digested with a total of 26 restriction enzymes. Thereafter, I separated the restriction fragments by agarose or polyacrylamide gel electrophoresis, a procedure that enables the fragments to be separated

according to their size. After staining the gels with ethidium bromide, I photographed them under UV light and compared the band patterns of the two dogs. Variation in the band patterns would indicate presence of RFLP. Despite an enthusiastic search, however, I failed to detect any RFLP in the tested pedigree. It is worthwhile to mention that this strategy has been highly successful in identifying RFLPs in pedigrees affected with other forms of PRA. Failure to detect RFLP in the *pd*-affected pedigree may be due to extensive inbreeding in the Miniature Schnauzers. Thus, there may be very little genetic heterogeneity in this breed.

Failure to detect RFLP in the *pd*-affected pedigree may be due to extensive inbreeding. . .

During the last couple of weeks that the program was in session, I adopted a different strategy aimed at identifying base changes between two test fragments by heteroduplex analysis. Unfortunately, time ran out; but I am returning home with the hope that this new approach will be successful.

I greatly enjoyed my research as well as the social aspects of the program this summer. It was a priceless and unique experience. I would like to thank all people from 'my' lab but especially Dr. Ray and Vicki Baldwin for their patience, humor and enthusiasm. Many thanks also to Linda Griswold and Josh Weisberg for doing an excellent job in coordinating the program and the Robert W. Woodruff Foundation for sponsoring me. Last, but not least, I am grateful to Dr. McGregor for his immense enthusiasm and dedication to the program.

Elizabeth Barnes - University of Illinois



My interest in veterinary medicine is both scientific and compassionate. While an undergraduate at Northwestern University, I completed a year long independent study investigating the effects of inhibin on FSH levels in the female rat. That research experience catalyzed my desire to pursue other research opportunities.

I will enter third year at the College of Veterinary Medicine in Urbana-Champaign this fall and intend to practice veterinary medicine upon graduation. The scientific aspect of veterinary medicine intrigues me, and I may find myself in graduate school in the near future. This program afforded an opportunity to participate in research again with a variation on the corn theme of Urbana! Obviously, the idea of a summer of research in a land of geographical contours had incredible appeal.

As a NIH and Merck Foundation Fellow, I was privileged to work in the Reproductive Physiology laboratory of Dr. Joanne Fortune. The laboratory is studying factors involved in the selection of ovulatory follicle, and the process of ovulation in the bovine.

The development of ovulatory size follicles occurs throughout the

estrous cycle in waves of follicular growth. By definition a wave includes three to six follicles that grow > 5 mm in size. An individual animal will either have 2 or 3 waves of follicular growth. During the cycle, the majority of follicles stop growing and one dominant follicle develops into the ovulatory follicle. The question of which follicle becomes dominant is still unanswered, however. The dominant follicle appears to be maintained by the negative feedback effects of its products on FSH levels, and its ability to mature towards ovulation in the face of lower FSH levels.

In the course of the summer I became involved in three projects concerned with the process of ovulation and the factors regulating the selection of the ovulatory follicle. During the process of ovulation, a preovulatory follicle undergoes several morphological and functional

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changes. Theca cells and granulosa cells that produce estrogen (responsible for heat behavior and ovulation) and inhibin become the corpus luteum with luteal cells that produce progesterone. Pre-ovulatory follicles were harvested on Day 8 (with Day 0 as day of standing heat) for in vitro studies. I assisted with the in vivo aspects by mapping daily follicular growth through rectal ultrasonography and assisting with surgery.

The second project involved characterization of the secretions of the bovine corpus luteum. PGF₂ α produced in the endometrium passes via the uterine vein to the

ovarian artery via a counter current mechanism. Prostaglandin is believed to induce regression of the corpus luteum. This theory, taken as dogma for years, has never been proven in the bovine. I assisted with surgeries fitting heifers with a microdialysis system designed to collect the secretions of a Day 11 or Day 17 corpus luteum.

Finally, I was involved in the development of a progesterone enzyme immunoassay (EIA) that, if feasible, would replace the standard progesterone radioimmunoassay (RIA). I collected jugular vein blood from which plasma samples were obtained to assay. I also monitored the heifers by ultrasound each day from Day 1 to Day 8 of the estrous cycle. The EIA is preferable to RIA because of the rising costs of radioactive waste disposal and the inherent safety of an assay that depends on enzymatically induced color change as the marker rather than a radioisotope.

This summer was a fascinating mixture of both clinical and research experiences. The hours I spent ultrasounding ovaries, collecting blood samples, and assisting with surgery added greater depth to my veterinary education. One aspect about research that I experienced time and again is that research is neither perfect nor predictable. Rarely does a cow "read the book" and have perfect follicular waves; and assays do not work every time! The time spent in the lab and in the barn added to my research experience and fueled my desire to consider a research career.

I am grateful to Dr. Joanne Fortune and her lab group for all their time and patience. To the folks in the program, I can't imagine 23 other people I would rather live with! I leave this program with mixed emotions and rich with knowledge, memories, and lasting friendships.

Philippa Beard - University of Sydney



During my summer at Cornell, I worked with Dr. Joel Baines on the herpes simplex virus, (HSV), a model for all animal herpesviruses. This involved using recombinant viruses, ensuring they are expressing the recombinant gene, and then testing the gene against radiolabelled DNA, using a DNA retardation gel assay. I learned a lot about working in a laboratory, what a research project involves, as well as different laboratory techniques.

The herpesvirus infects a cell, enters the nucleus, turns off cellular DNA replication, and uses the machinery to replicate more of its own DNA and proteins. An important part of the life cycle of HSV is the cleaving of its DNA strands into genomic sizes so they can be packaged into virions. HSV has a group of proteins that recognize the DNA sequence at the cleavage site, attach here, and cut the DNA strands. My working hypothesis was that at least one of these proteins is a DNA binding protein.

The HSV genome is made up of a number of genes. I studied UL6, UL15, UL28, UL32, and UL33 which have previously been shown to be required for HSV DNA cleavage/packaging. Our goals were to place these genes into the baculovirus

genome, obtain expression of the HSV gene, and test the recombinant proteins for DNA binding activity.

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It is important to ensure that the recombination has worked, and that the baculovirus is actually expressing the inserted HSV gene. To do this, I employed two different techniques. I used a Western blot to determine if the UL28 protein was being expressed by the recombinant baculovirus. Protein produced by the baculovirus was immobilized onto a nitrocellulose membrane, and reacted with UL28 protein antibody. In the recombinant lanes, the antibody bound specifically to a band not seen in the normal or wild type baculovirus lanes. This meant that UL28 was being expressed by the recombinant baculovirus.

The other technique I used involved a Southern Blot. I made a radioactive strand of DNA that was complementary to the UL28 and UL32 genes, and used them to probe purified recombinant baculovirus DNA immobilized on nitrocellulose membranes. Upon autoradiography, novel bands were visible that represented hybridization of the UL28 and UL32 genes with the DNA probes in the recombinant baculovirus.

Once I had recombinant baculoviruses that I was confident were producing HSV protein, I ran a DNA gel retardation assay. I used DNA sequences known to be cleaved during DNA packaging as a probe. The probe was purified and

radiolabelled, then reacted with a nuclear extract of recombinant and wild type baculoviruses. The reactions were then separated on an acrylamide gel, followed by autoradiography to locate the radiolabelled DNA probe. When compared to lanes containing uninfected or wild type baculovirus nuclear extract, the lane containing recombinant nuclear extracts should reveal a new band, corresponding to the HSV protein binding to the radiolabelled probe. Appearance of such a band would suggest that the HSV protein functions as a DNA binding protein. I intend to confirm that supposition by using antibody to the HSV gene product. Antibody added to the binding reaction should cause the protein-DNA complex to migrate more slowly, shifting its location in the gel.

So far, my results have been encouraging, but not conclusive. I am confident that varying the reaction conditions will produce a positive result.

I came to Cornell not knowing anything about working in a laboratory, and with no sure future mapped out. One of the most important things I learned is how many different career options there are for individuals who have a veterinary degree. The time I have spent working for Dr. Baines has shown me that research is much more interesting and rewarding than I had imagined. I am now seriously considering pursuing a research degree once I have completed my veterinary studies.

I would like to acknowledge the Mellon Foundation, and the Queen's Trust for their financial support. I would also like to thank all the people in my lab for all their time this summer, and most of all, Joel Baines, for his help and infinite patience, which helped make my summer so enjoyable and rewarding.

Adrienne Bentley - Cornell University



Although for most of my life I had wanted to become a veterinarian, I became sidetracked in college by a fascination with neuroscience research. Over several years, I investigated central nervous system regeneration in goldfish. This project combined the use of delicate neurosurgical techniques with histological and morphological studies of neuronal regeneration. As Cornell University has a very good D.V.M./Ph.D program, I was delighted to be accepted here, seeing it as an opportunity to pursue my interest in becoming a veterinarian as well as continuing research. Having finished my first year at Cornell vet school, the summer Leadership Program provided a welcome chance to return to research.

I participated in a research project in Professor Rick Cerione's lab, working under the direct supervision of Dr. Shubha Bagrodia. Work in this lab has focused on the roles of various cellular proteins in the regulation of cell growth. Cells receive external signals from their environment which can cause growth by eliciting changes in gene transcription. One of the main types of signaling from the cell surface to the nucleus is through protein ki-

nase cascades, eventually leading to activation of transcription factors, alteration of gene expression and changes in cell growth. Cell growth can then be regulated by activation or inactivation of signaling pathway elements, often initiated by a GTP-binding protein which acts as a switch. This is an area under intense investigation, because many of these signaling elements, when mutated, become oncogenes that cause uncontrolled cell growth.

My project focused on a signaling pathway involving the GTP-binding protein Cdc42, which is known to be involved in alterations in the actin cytoskeleton that are important for changes in cell growth. It has been found that Cdc42 activates a serine-threonine kinase, PAK. PAK may initiate cascades that are responsible for mitogenic responses to growth factors, and for cytoskeletal changes. One member of the PAK family, mPAK-3, was isolated by Dr. Shubha Bagrodia, with whom I was working. During the summer, we set out to examine the effects of different mutated forms of mPAK-3 on cell growth.

All members of the PAK family have two highly conserved domains which must be vital for their normal function. One is a Cdc42 binding

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domain at the amino-terminal end, and the second is a kinase domain at the carboxyl-terminal end. By making mutations in these domains, we altered the activity of PAK. First, constitutively active PAK was made by making mutations in the highly

conserved Cdc42 binding domain. By altering the Cdc42 binding domain, the interaction of this domain with the kinase domain was disrupted, thus activating PAK. A second constitutively active PAK was also made, this time by altering the kinase domain such that an autophosphorylation event that helps activate PAK was mimicked. The third type of mutation made was a kinase dead mutant, in which the ATP binding site was mutated so that PAK could not phosphorylate and activate its target. This is called a dominant negative mutant, because it will bind Cdc42, but will not promote signal transduction.

To prepare these mutants, a small piece of the PAK gene containing the desired mutation was made using the polymerase chain reaction (PCR). Restriction endonucleases were then used to cut the ends of the PCR product, and to generate a plasmid expression vector so that the mutated DNA could be spliced into the plasmid. After introducing the DNA insert into a plasmid, the vector was transformed into E.coli, and DNA from colonies was screened by gel electrophoresis to determine if the desired plasmid had been created. If so, the vector was used either to generate stable cell lines, or in focus-forming assays, which can indicate whether expression of DNA in a cell has altered the growth properties of the cell. As we had some difficulty in making several of the mutants, we have just recently started focus forming assays and stable cell lines, and should be obtaining results from them in a few weeks.

Overall, I feel very satisfied with my experience this summer. Not only did I become familiar with a variety of molecular biology techniques and concepts, but I also gained a more realistic view of the normal pace of research.

Kate Creevy - University of Tennessee



I have always known I wanted to be a veterinarian—that is until my first year of veterinary school. After an undergraduate career at Georgetown University, which combined a Bachelor's Degree in Biology and thesis research in animal behavior, with theater, politics, and a great deal of travel, I found myself inexplicably frustrated by the first year of my veterinary education at the University of Tennessee. I learned of Cornell's Leadership Program and was instantly attracted to a program designed for veterinary students interested in research, but undecided upon a career path. I applied, was accepted, and pined my hopes for answers on this summer.

I've spent the past ten weeks researching the role of splanchnic innervation in adrenal secretion of cortisol in fetal sheep, with Tom McDonald, PhD, of Cornell's Laboratory for Pregnancy and Newborn Research. We surgically exposed and transected the splanchnic nerves supplying the adrenal glands of late-gestation fetal sheep, and subjected the recovered sheep to a chronic hypoxic stress. This physiological stress induces pituitary release of ACTH which, in turn, in-

duces adrenal secretion of cortisol. In normal fetal sheep, under long-term hypoxemia, ACTH levels decline nearly to baseline after approximately twelve hours. However, cortisol levels remain elevated, suggesting an additional control system for cortisol release. We theorized splanchnic nervous involvement in this regulation, and hypothesized that our splanchnicotomized fetuses would exhibit lower circulating levels of cortisol than the control group when subjected to the same environmental stress. In fact, this is what we found.

...cortisol levels remain elevated, suggesting an additional control system for cortisol release.

The significance of our project arises from the responsibility of fetal cortisol for organ maturation and the timing of parturition in sheep and other ruminants. By improving our understanding of the development and control of the adrenal glands, we increase our ability to predict, and perhaps to alter, the timing of birth in a variety of species. Despite the complexity of the protocol, the intensive labor hours, confusing data and tedious statistical analysis, my active involvement was so excitingly different from the passive fact-assimilation expected of a first-year veterinary student that I was not discouraged.

I also spent the past ten weeks discussing and debating ethics, career options and the meaning of leadership. I met fascinating veterinarians whose career paths have been wonderfully winding. I've seen how easily I could pursue clinical interests, then change focus and location, entering academic research when I find the right question. I've realized the creativity, devotion and

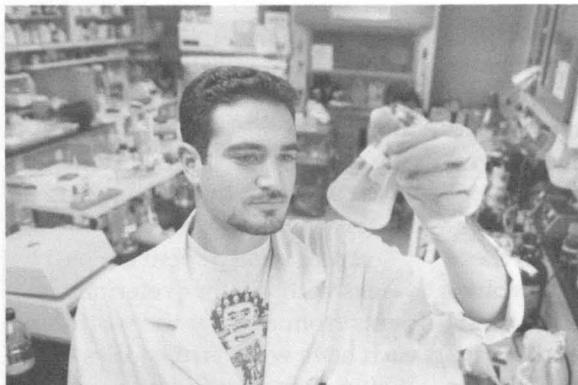
skill essential to a successful experiment. This is my kind of veterinary medicine—physical and intellectual mobility, creative opportunity, and the necessity to utilize the persistence that a lifetime of juggling activities has taught me.

And I've also spent the past ten weeks with 23 other veterinary students from all over the world who each have wonderful stories to tell. We've exchanged ideas about our histories and our futures, and traveled together to places we might otherwise never have seen. I've been thrilled to meet peers who approach our profession by eagerly engaging in constant, active pursuit of knowledge. My colleagues here are competent veterinary students, who also have dimensions outside that sphere, and support my need to blend many interests with my professional goals.

I have found answers, and I will return to my second year of veterinary school in Tennessee with the knowledge that like-minded peers exist, both in school and established in the profession, and that it was the neglect of my many dimensions, not the curriculum itself, which led to my frustrations. My rediscovery of the limitless options within veterinary medicine will enable me to approach my second year with renewed dedication, and to prioritize a variety of interests at equal levels with classroom education. Involvement in the Leadership Program has helped to show me a niche that I can carve into the profession I have pursued for so long.

Naturally, this summer would not have been possible without the involvement and assistance of the NIH and Merck Foundation, Tom McDonald, Peter Nathanielsz, and the LPNR, UT's Dean James Brace, my family, my fellow Fellows, and the four Career Day counselors whose insight was so valuable, honest, welcome and so very well-timed.

Demian Dressler - Cornell University



I was first exposed to research during my undergraduate years at the University of California at Davis, where I majored in Animal Physiology as a prelude to veterinary school. During this period I decided that I would like to continue exploring this area, as I viewed research as a potential career option. When the opportunity to apply for the 1995 Leadership Program presented itself, I decided that this would be an ideal way to accrue further experience in basic scientific research and its applications. I was delighted that I was delegated a space in the program.

The majority of my time this summer was spent in the laboratory of Dr. Colin Parrish. I was able to perform my own research in the dynamic environment of the Baker Institute, and through consults with colleagues and journal club meetings had the opportunity to interact with members of the scientific community elsewhere at Cornell. In this way I was able to get a well-rounded picture of this stimulating occupation and lifestyle.

My research project focused on parvoviruses and the infections caused by these agents. Parvoviruses cause leukopenia and life-threatening diarrhea in several animal species. Although effective vaccines exist, the precise mechanisms of infection in permissive cells remain unclear. The question we were investigating was the location

of viral uncoating, or the disintegration of the protein coat which encloses the viral genetic material, in infected cells. This process is necessary for all subsequent steps leading to the generation of new virus particles. More specifically, we were interested in whether

capsid disassembly occurs at the plasma membrane or at some region beyond this boundary, such as the cytoplasm. The answer to this question is not only interesting in itself, but it might be relevant to other viral systems.

The method used in addressing the central question involved the intracytoplasmic expression of a gene coding for a single chain protein product with sequences identical to the variable regions of the heavy and light chains of a mouse anti-CPV IgG, A3B10. Our working premise was that the protein, called 535, would fold within the cytoplasm in such a way that a binding site would be generated, capable of binding three adjacent capsid proteins of CPV. If CPV infects cells expressing 535 such that the capsid is still intact in the cytoplasm, 535 would adhere to this capsid and stabilize it, blocking capsid disassembly. Since the viral nucleic acid would be unavailable to the host cell's enzymatic machinery, and viral replication could not take place, new virions would not be produced. The result would be intracellular viral neutralization. On the other hand, if capsid disassembly occurred at the plasma membrane or within an endocytic vesicle, one would not see a decrease in the rate of CPV infection of permissive cells.

In my initial studies, I used a prokaryotic system before attempting expression in eukaryotic cells. I

used agarose gel electrophoresis to sequence across junction regions of the expression vector, pMAL-p2, containing the insert which codes for 535. The sequences matched our predicted ones, and thus I was sure that the insert-vector ligation had occurred precisely. I then induced

The sequences matched our predicted ones, and thus I was sure that the insert-vector ligation had occurred precisely.

E. coli to express 535 using IPTG, but was unable to detect the new protein using polyacrylamide gel electrophoresis. I next performed affinity purification to separate 535 from other bacterial proteins in case expression was occurring but at low levels. 535 was not seen after affinity purification. We hypothesized that the form of 535 produced using the pMAL-p2 expression vector was toxic to the bacteria producing it. Therefore, I spliced the insert into two new expression vectors, pFLAG and pMAL-c2, which hopefully will be capable of generating forms of 535 lacking this putative toxicity.

Through working with Dr. Parrish I was exposed to many different techniques encountered in molecular biology, en route to those small successes which make the time spent in the lab quite satisfying.

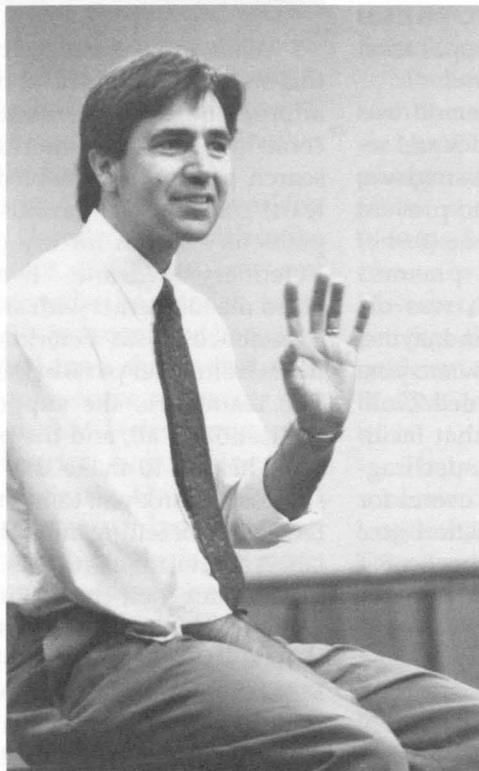
I would like to thank the NIH and the Woodruff Foundation which supported my work and made it possible for me to participate in this enriching program. Additionally, many thanks to Dr. Colin Parrish whose relentless motivation and patient instruction will continue to serve as an inspiration to me and others around him. I would also like to thank Dr. John Parker and Wendy Weichert for their help.



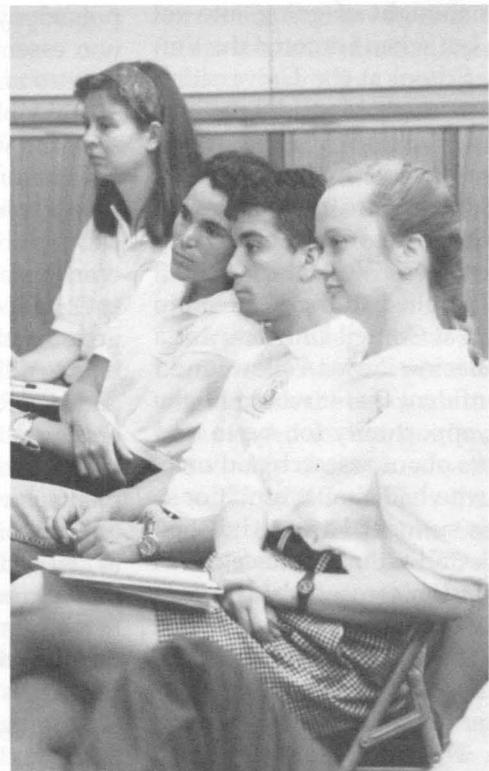
Joshua Weisberg served as program coordinator for the 1995 program.



Dr. Gerry Hickey and Dean Robert Phemister. The Merck Foundation has sponsored the Leadership Program in each of the past six years.



Dr. Jim Flanders addressing the students at a "Career Day" meeting.



Gerti Altreuther, Maria Fuente, Ross Koenig and Elizabeth Adkins participated in discussing career options for veterinarians.

María Fuente - University of Madrid



I have always wanted to be a veterinarian. Since I was a kid I kept dogs, cats, birds, rabbits and all kinds of animals at home. They helped me decide my future: I wanted to work with animals, treat them if they were sick and take care of them. I never thought of going into research, but when I entered the Veterinary School at the Universidad Complutense de Madrid, I realized that there were many other aspects of the profession that were open to me and I wanted to explore them. I was in the process of completing my fourth year of Veterinary School when I applied to the Leadership Program at Cornell University as a La Fundación Purina Fellow, and I was confident that it would be the perfect opportunity for me to find out more about research and meet others who had similar ambitions.

This summer I was privileged to work under the guidance of Dr. Schat and his research assistant Priscilla O'Connell in the Department of Avian and Aquatic Animal Medicine (now part of the Microbiology and Immunology Department). The principal task was to construct a recombinant Marek's Disease Virus (MDV) expressing Interferon (IFN) gene. Marek's Dis-

ease (MD) is an economically important disease of chickens caused by MDV, an oncogenic Alpha herpesvirus, which causes immunosuppression and T-cells lymphomas. IFN is a glycoprotein, in my case produced by fibroblasts, that enhances host cell resistance to certain tumors and viruses.

Recombinant MDV strains can be constructed by homologous recombination, in which total MDV DNA is transfected into susceptible cells together with a transfer vector. A transfer vector contains two MDV DNA fragments with the gene of interest inserted in between the two MDV DNA fragments. During the replication process of the virus, the transfer vector replaces the original fragments of viral DNA. We used the transfer vector pBSL1pSV40 to insert the IFN gene. In this vector, a part of ORF L1 has been replaced by the SV40 early promoter, a multicloning sequence and a polyadenylation signal. ORF L1 is non essential for MDV replication *in vitro* and *in vivo*.

The pBSL1pSV40 plasmid was digested with EcoR-I and Sma-I restriction enzymes. The plasmid was then dephosphorylated to prevent self ligation. The IFN gene (0.9kb) was obtained from a plasmid (pCH132,4109bp) which was digested with XbaI restriction enzyme, blunt-ended with Klenow enzyme and an EcoR-I linker was added. Linkers are oligonucleotide that facilitate the cloning of blunt-ended fragments, but they are also useful for "introducing" new restriction sites at desired positions. The EcoR-I linker contains a specific recognition sequence for EcoR-I. I digested the plasmid with EcoR-I and Sma-I restriction enzymes. The EcoR-I and Sma-I fragment containing the IFN ORF was ligated into the EcoR-I — Sma-I digested and dephosphorylated pBSL1pSV40. I transformed this ligation reaction with *E. coli* com-

petent cells, strain DH5alpha, and plated onto LB ampicillin plates. Colonies which grew were screened by "In Well Lysis" method, and once recombinant colonies were identified, they were grown overnight and the plasmids were extracted. To verify these colonies were correct, the plasmids were digested with EcoR-I and Sma-I restriction enzymes and were electrophoresed on a 1% agarose gel.

I managed to obtain a colony which contained the recombinant plasmid with the expected 0.9Kb IFN gene. The plasmid that I made will be used to construct a recombinant MDV expressing IFN gene.

I managed to obtain a colony which contained the recombinant plasmid with the expected 0.9Kb IFN gene.

What an incredible experience this summer has been! It not only afforded me the opportunity to become involved in an interesting research project, but enabled me to learn more about various career paths as I search for my place in Veterinary Medicine. It also enabled me to interact with and share experiences with veterinary students from other parts of the world. The teamwork, the support and dedication of all, and the program itself helped to make this a huge success. Thank you to my mentor, Dr. Schat, for letting me work in his lab. A special thanks to Priscilla and Rahman for their cheerfulness, patience and support of my project. I will always be indebted to Dr. McGregor for making this program possible, and thank you Linda Griswold and Joshua Weisberg for your dedication to it.

Rachael Gray - University of Sydney



I am in my fourth year of Veterinary Science at the University of Sydney. Ever since I can remember, I have aspired to become a vet, particularly in the hope of doing some form of research involving marine mammals. For this reason, I applied for Veterinary Science and was accepted. I saw the Leadership Program as a wonderful opportunity to gain valuable research skills as well as to see and experience another part of the world.

I spent the summer working with Dr. Dorothy Ainsworth in the Department of Clinical Sciences. Previous studies in our lab using chronically instrumented horses on a treadmill have shown that with increasing exercise intensity, diaphragmatic EMG (electromyogram of the electrical events in muscle) increases linearly. However, when there is resistance to breathing such as laryngeal hemiplegia, an incremental increase in exercise intensity reduces diaphragmatic EMG. Two hypotheses could explain this finding. The first is that the diaphragm may be neurally inhibited in an attempt to prevent fatigue. The second is that end expiratory diaphragmatic length (EEDL) changes may be responsible for "artificial"

changes in the EMG.

Our aim this summer was to measure EEDL as well as phasic length changes of the diaphragm during control and inspiratory resistive loading (IRL) trials. To do this we implanted sonomicrometers into the diaphragm of subject horses. Sonomicrometers are ultrasound transducers which consist of two piezoelectric crystals coated with resonating material. The first crystal acts as a transmitter, whilst the second is a receiver. The distance between the crystals and hence muscle length can be determined on the basis of the transit time of ultrasonic waves propagating from one crystal to another. We successfully implanted sonomicrometers into two horses. The "sonos" were validated in vitro and in vivo. EMG electrodes were also implanted in the diaphragm and the external intercostal muscle to determine if the latter muscle is recruited in the IRL trials in order to spare the diaphragm from fatigue.

Preliminary data has shown that with increasing exercise intensity, as electrical activity of the diaphragm increases, the degree of phasic shortening also increased. We also found that EEDL also increases with increasing intensity. However, we have no evidence as yet that these length changes are sufficient to explain the decrease in the EMG observed in previous IRL trials.

It appears from our studies that sonomicrometry is responsive to length changes in vivo and in vitro. They also appear to measure length changes during exercise. Future studies will employ sonomicrometers to measure diaphragmatic length changes under control and IRL conditions. If EEDL does remain unchanged and phasic shortening is decreased in the IRL trials, it would point to a central medullary inhibitor of neurological input

to the diaphragm.

This is the first instance in which sonomicrometers have been implanted in the awake, exercising horse. They are expected to provide valuable information regarding respiratory function in the horse and the role of the diaphragm at higher intensity exercise levels.

This is the first instance in which sonomicrometers have been implanted in the awake, exercising horse.

I found my time at Cornell extremely worthwhile and I had a wonderful stay here. I gained valuable skills in research and in many aspects of clinical care. It was extremely rewarding for our lab when we finally got a sono signal in an awake, exercising horse as this technique has not been used before in horses but can now be employed in future studies. I also had the opportunity to travel this summer which was one of the great side benefits of the program. The best and most valued aspect of the summer however, was the friendships and experiences that I shared with the rest of the LP students who were a great group of people.

I would like to thank my mentor, Dorothy Ainsworth, for the challenging task she set us for the summer as well as the guidance and knowledge she was so willing to share. In addition, many thanks go to Kim Snedden, Mike Santiago, Meg Nalevanko and Beth Davidow. I would also like to thank the treadmill staff for all their help with the horses.

Finally, I would like to thank Dr. McGregor, Linda Griswold and Joshua Weisberg for their role in organizing the program as well as the Robert W. Woodruff Foundation for its support of my activities.

Danielle Greenberg - The University of Liverpool



"All animals except man know that the principle business of life is to enjoy it, and they do enjoy it, as much as man and other circumstances will allow." SAMUEL BUTLER, 1903

For me these sentiments strike a deep chord, for they express the dyadic goals of my career plans for as long as I can remember; to enjoy life and to do so by helping animals enjoy theirs.

Since completing my undergraduate degree in History at Oxford University, I worked at the House of Commons in London, and spent over a year at the University of Pennsylvania Small Animal Hospital. I am now entering my third year at Liverpool Veterinary School.

Having expressed an interest in the field of reproduction, I was delighted to learn that I would be working with Dr. Vicki Meyers-Wallen whose research interests are inherited disorders of the reproductive tract. As my summer project, I primarily investigated *Sry*-negative XX sex reversal in dogs.

Sex reversal occurs when chromosomal sex (XX or XY) does not agree with gonadal sex (ovary or testis). In our case, it refers specifically to XX animals with a normal

female karyotype that develop testicular tissue to varying degrees.

Having established a recessive inheritance pattern for the trait within a colony of American Cocker Spaniels (ACS), the real question remained: How could an animal lacking a Y chromosome develop testicular tissue? So far the only established cause of such XX sex reversal has been a Y-to-X translocation of the *Sry* gene. Considerable evidence suggests that the Y-linked *Sry* gene is the normal genetic switch for testis differentiation. Specifically, *Sry* encodes a DNA binding protein of the high mobility group (HMG). This HMG box is conserved between species, and mutations are associated with failure of testis development.

In previous studies, Dr. Meyers-Wallen established a protocol with which to screen the genomic DNA of affected dogs for the *Sry* HMG box using canine-specific primers. Her results on groups of ACS and German Short-Haired Pointers showed no signs of *Sry* in the affected dogs, leading her to conclude that a mutation in an autosomal gene downstream of *Sry* was the probable cause.

Samples from affected dogs included a Basset Hound, a breed in which the condition had not yet been reported, and a hermaphrodite horse which had never previously been studied. This presented a new challenge; would they show a *Sry* translocation? It was to be my job to find that out!

I began by extracting DNA from the blood samples, and established the concentration using UV spectrophotometry and gel electrophoresis. In addition to running PCR with canine *Sry* primers, I ran a parallel test using primers for canine HPRT, a "housekeeping" gene which could be expected to show a product in all animals irrespective of sex. I used a normal male and female dog as posi-

tive and negative *Sry* controls, and a "no-template" control without DNA to guard against contamination of the reaction mix. The normal dog controls came from Labradors, a breed in which XX sex reversal has not yet been documented.

I used gel electrophoresis to separate my PCR products according to weight/numbers of base pair products, stained with Ethidium Bromide, and took photographs in UV light.

My results showed the predicted HPRT bands for all animals at 177bp, but only the normal male showed *Sry* bands at 104bp. Using similar conditions, with normal male and female horse controls and consensus *Sry* and HPRT primers, I found similar results.

My results showed the predicted HPRT bands for all animals at 177bp, but only the normal male showed *Sry* bands at 104bp.

It appeared that these animals were also *Sry*-negative XX sex reversed. Studies are now being increasingly focused on mutations in genes downstream of *Sry* in the testis differentiation cascade, particularly the *Sox 9* gene which contains an *Sry*-like HMG box and has been shown to be expressed in cells before differentiation occurs. Possibly a mutation in the *Sox 9* HMG box causes activation and hyperfunction of the *Sox 9* protein causing testicular tissue to develop.

I am indebted to Dr. Meyers-Wallen for her patience, generosity, knowledge and sense of humor. My sincere thanks to the Wellcome Trust for supporting my fellowship; this has been a summer I shall never forget!

Marcus Gunew - University of Sydney



In 1990 I enrolled at the University of Sydney in the Faculty of Veterinary Science, and am currently nearing the end of my final year. During 1993 I took a year off my veterinary studies to conduct research and to fulfill the requirements for a Bachelor of Science (Veterinary) degree. During that year, I worked in the department of Veterinary Pathology in the field of immunology.

I have spent this summer as a Mellon Foundation Fellow working with Dr. Gleed and his associates in the area of equine physiology. My project involved measuring pulmonary artery blood temperature in the non-exercising athletic horse. We hypothesized that arterial blood temperature fluctuates in relation to skin blood flow or rate of respiration. To test this hypothesis, we measured pulmonary artery blood temperature in real time, and correlated the results with skin blood flow and respiration rate.

Core temperature fluctuations have been recognized previously in the horse, first in 1987 by Thiel et al., while measuring rectal temperatures in the walking horse, and later in this laboratory, in 1994 (unpublished). Core temperature fluctua-

tions seem not to have been reported in other species.

There are four mechanisms by which animals can exchange heat with their environment: 1. Radiation of infra-red energy; 2. Convection, both as blood movement within the animal and to fluids and gases in the environment; 3. Conduction to objects in direct contact, and 4. Evaporation, primarily either as sweat or respiratory tract evaporation. The horse at rest can rapidly effect respiratory evaporation by a change in respiration rate or distribution of blood flow.

Our study was conducted in five Standardbred horses. All were confined in stocks. A custom made catheter with a thermistor for measuring temperature in the pulmonary artery was used. The probe was positioned via an introducer in the cervical jugular vein and advanced through the heart into the pulmonary artery. The position of the probe in the pulmonary artery was confirmed by observing the characteristic blood pressure waveform at the probe tip.

Skin blood flow was measured at the cranial edge of the scapula, using an ALF21 laser doppler unit. The area to which the probe was attached was shaved and cleaned. Respiration rate was determined by measuring respiratory excursions. This was accomplished by securing a set of pneumobellows around the point of maximum respiratory excursion and recording pressure fluctuations.

All data were collected by a computer using the ASYST data collection and analysis program at a sampling rate of 32 hertz.

Satisfactory measurements were made in four of the five horses; technical difficulties were experienced with the fifth subject. Measurements made in the four horses were not significantly different in regard to the frequency or magni-

tude of temperature fluctuations. The pooled results are shown in the accompanying table.

	Mean	SEM	Range
Period (sec)	58.6	2.8	10.5 - 199.5
Change (°C)	0.19	0.014	0.05 - 0.68

Table 1: Results from pooled data.

The analysis required to determine whether pulmonary blood temperature correlates with skin blood flow or respiration rate will probably involve Fourier transformation, and has not yet been completed.

The observed variation in body core temperature is relevant to other work conducted in Dr. Gleed's laboratory involving measurements of lung water. The diffusible indicator used for this purpose is heat. Thus, variations in pulmonary blood temperature can be expected to complicate data analysis.

I found this summer to be an exceptional experience, not only as an opportunity to further my research experience but also as a way to meet veterinary students from around the world. I would like to thank everyone who helped make the program possible but especially the Richard King Mellon Foundation for its sponsorship. I also wish to thank my research mentor Dr. Gleed, and Dr. Pam Wilkins without whose help my project would not have been possible.

Krista-Britt Halling - Ontario Veterinary College



As a student entering my second year at the Ontario Veterinary College, University of Guelph, I have long been intrigued by the diversity and challenges that research could offer. In an effort to investigate this aspect of veterinary medicine, I have been involved in equine pulmonary function tests and canine cardiology research, as well as population ecology studies on turtles, biological research in Costa Rica, and giant panda conservation efforts in China. It was this interest in pursuing post-graduate work in veterinary medical research, coupled with my involvement in student government and university affairs, that attracted me to Cornell's Leadership Program.

As a Mellon Foundation Fellow, I had the privilege of working with Dr. Drew Noden in the Department of Anatomy. I conducted research on the embryonic origins of tongue and laryngeal muscles, using chicken embryos as a model system.

The normal function of an organ depends on proper integration of its muscle, neural, and connective tissue components. Specific embryonic interactions are required for the development of these correct spatial relations and tissue architec-

ture. Analysis of muscle cell lineages provides an understanding of the origins, movements, and eventual segregation of myogenic populations during development.

My project had two goals: 1) to catalogue the contributions of each of the first five somites to developing muscles, cartilages, and blood vessels, and 2) to determine whether precursors of individual tongue and laryngeal muscles are segregated in specific somites or distributed among several.

Lineage analysis of these muscles was achieved by micro-injection of an avian retrovirus into specific embryonic somites at stage 9 (7-somite stage). The modified, replication-incompetent spleen necrosis virus incorporates a *lacZ* gene into the genome of infected somite cells, resulting in expression of a bacterial β -galactosidase enzyme in all daughter cells. Spreading throughout the cytoplasm, the enzyme creates a distinct blue labeling upon exposure to a substrate. All progeny of infected precursor cells can then be readily identified in whole mount and serial sections.

After sanding a circular window in the egg shell, the embryo was lightly stained and staged according to the Hamburger-Hamilton staging series. A solution containing the retrovirus was injected into a somite using a picospritzer. The window was sealed with tape and the egg incubated until the embryo was removed for dissection at 9 days of age.

Of the 118 embryos injected, 62 survived to 9 days of incubation. Twelve of these survivors contained labeled cells. Somites 1 and 2 preferentially labeled laryngeal muscles, while injections into somites 2-5 labeled intrinsic and extrinsic tongue muscles. All of these somites contained precursors of epaxial and hypaxial muscles. Each of these somites also contained precursors

of specific regions of the occipital region of the skull, and chondrogenic precursors in somites 1 and 2 also formed the laryngeal cartilages. Finally, each somite contained progenitors of endothelial cells. In contrast to the limited dispersal of myogenic and skeletogenic precursors, angioblasts move extensively throughout the head and neck regions, seeding axial, visceral, and subcutaneous tissues over a wide area.

These data indicate that progenitors for specific tissues behave quite differently. Skeletal precursors develop segmentally, with each somite giving rise to a separate part of the skull. Muscle precursors develop regionally, with groups of muscles derived from several adjacent somites. The finding that each somite contains precursors of many tongue and laryngeal muscles indicates that there is no strict segmental segregation of muscle progenitors. Blood vessel precursors scatter widely and intermingle with angioblasts from many other somites.

...progenitors for specific tissues behave quite differently.

I found the Leadership Program extremely rewarding. Although intense, there is enough flexibility in the program to balance research commitments with personal growth and exploration of new locations. I greatly appreciate the Mellon Foundation's support for my participation in the program, and would like to thank the program organizers for doing such a terrific job. I am especially grateful to Dr. Drew Noden and members of his laboratory for the sincere interest, guidance, and enthusiasm they displayed throughout my project.

Wendy Harrison - Royal Veterinary College



A small skewbald Shetland pony with sarcoids was probably responsible for my entry into the veterinary profession. I enrolled in the Royal Veterinary College London in 1990 with the sole intention of becoming a practicing veterinarian. But, after my second year, I decided to broaden my horizons by applying to intercalate a B.Sc. at King's College London. In my dissertation I investigated the ionic requirement for capacitation of mouse sperm. I thoroughly enjoyed this first experience of research. In 1993, I was lucky enough to be selected as a member of the RVC Undergraduate Research Team. We spent 10 weeks working in conjunction with the University of the West Indies in Trinidad on the epidemiology of *Trypanosoma cruzi*. This furthered my interest in the process of research. When I learned about the Leadership Program at Cornell, I saw it as a unique opportunity to further explore this area of veterinary medicine and the opportunities offered to veterinarians in research careers.

I had the pleasure of working in Dr. Suarez's laboratory in the Anatomy Department. Dr. Suarez's interests lie in sperm motility and transport in the female reproduc-

tive tract. The project that I conducted under the direct guidance of graduate assistant, Dr. Rejean Lefebvre, was initially concerned with characterization of sperm binding to bovine oviductal epithelium. In the cow, the onset of estrus and ovulation may be separated by nearly two days and insemination may occur at any time during this period. The sperm must be maintained in a state where they can realize their fertilizing potential at the time of ovulation. In addition, an ejaculate may contain millions of sperm and there must be a mechanism that regulates the numbers of sperm that reach the oocyte. In many species, it has been shown that spermatozoa are temporarily arrested in the initial part of the oviduct, forming a reservoir. It is thought that structural components, entrapment by mucus, and sperm binding to the epithelium, may all be important in the creation and regulation of this reservoir. Oligosaccharide groups may be implicated in mediating the binding of the sperm to the oviductal epithelium. Our study set out to determine the nature of this binding.

During the experiments, we harvested oviductal epithelium from preovulatory heifers. The animals were ovariectomized and the oviductal epithelia from the ampulla and isthmic regions were collected. The cells were allowed to incubate. During this time, they formed small vesicles called explants. Explants were incubated with a number of glycoproteins and monosaccharides in the presence of sperm. The number of sperm bound to each explant was determined by analyzing videotapes. The images were then digitized and the density calculated. Initially, a number of glycoproteins with different terminal monosaccharides were selected that are important in other carbohydrate-mediated cell adhesions. We dis-

covered that fucoidan, a compound with predominantly sulfate fucose moieties, significantly inhibited sperm binding.

. . . fucoidan . . . significantly inhibited sperm binding.

To ascertain which part of the fucoidan molecule was responsible for this inhibition, sugar moieties containing sulfated groups and fucose monosaccharides were tested. It appears that only fucose significantly inhibits the adhesion. Further study of the dose response to fucose was carried out and the results suggest that fucose acts as a competitive inhibitor and a fucose moiety may mediate sperm binding *in vivo*.

I would like to thank Dr. Suarez who has been such an enthusiastic and knowledgeable mentor and a source of inspiration. I also owe a debt of gratitude to Dr. Rejean Lefebvre for his unending patience and guidance and his great sense of humor, without which my summer would not have been so enjoyable! I must offer sincere thanks to my sponsor, the Wellcome Trust, and particularly Dr. Patricia Chisholm for her interest.

I have had an exceptional experience here at Cornell on many levels. It has been a very rewarding experience to be involved in research at such a well-respected establishment and with such a varied and interesting group of international students. I would like to thank Dr. McGregor, Linda Griswold, and Joshua Weisberg for all the hard work they have put into making this program such a success. I think this exposure to the varied opportunities available to veterinary graduates is most valuable, and I feel privileged to have been a part of it.

Christine Harshbarger - University of Pennsylvania



Prior to veterinary school, I completed a BA in Biology at Swarthmore College in 1993. I am currently entering my third year in the veterinary school at the University of Pennsylvania. At Penn, I have managed to expose myself to a broad range of veterinary related experiences. I volunteered in the wildlife service for the last two years and participated in the colic team for a year. During the school year I work in the Medical Genetics Department, where my duties involve animal health care, preventative medicine, artificial insemination, and puppy and kitten socialization. Last summer I began a research project in Medical Genetics at Penn. After veterinary school, I would like to complete an internship and eventually a residency in Pathology, Medical Genetics, or Reproduction. I may eventually pursue a graduate degree as well.

Thanks to the Richard King Mellon Foundation, I was able to work on a project in the Comparative Hematology Section of the Diagnostic Laboratory (CHL) under the guidance of Dr. James Catalfamo. We attempted to determine the feasibility of developing a serum ELISA to screen for von Willebrand's dis-

ease (vWD) in dogs. von Willebrand's disease is a heterogeneous group of bleeding diatheses that result from quantitative or qualitative changes in von Willebrand factor. It is the most common hereditary bleeding disorder in man and dogs. Unlike the hemophilias, it has an autosomal mode of inheritance, so both males and females are affected. vWD was first recognized in humans in 1924 by Dr. Erich von Willebrand in Finland, and in dogs in the 1970's by Dr. Jean Dodds in the US. The disease has been recognized in 54 breeds of dogs to date.

von Willebrand factor is an important protein in primary hemostasis. It is essential for the attachment of platelets to the subendothelium exposed during vascular injury. This attachment of platelets to the site of damage leads to platelet plug formation and primary hemostasis. von Willebrand factor (vWF) has a region that interacts with the subendothelial collagen. Once bound to the subendothelium, another region of the protein is able to interact with a receptor on the surface of platelets. This promotes adhesion of platelets to the subendothelium of damaged blood vessels and subsequent activation of the platelets.

Several diagnostic tools can be utilized to aid in the diagnosis of vWD, but the most common methods measure plasma vWF antigen (vWF:Ag). Most forms of vWD in dogs result in a decrease in vWF:Ag that can be quantified by several tests. The test that the Cornell CHL uses is a double sandwich enzyme linked immunosorbent assay (ELISA) that quantifies plasma vWF:Ag.

The purpose of my study was to determine whether the assay could be modified to measure serum vWF antigen in dogs. Serum is the fluid phase of blood after clotting has

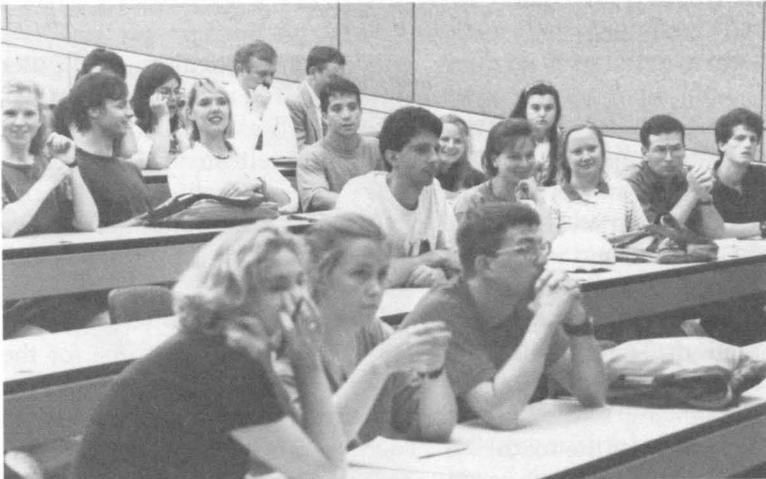
occurred. According to a previous study, the level of vWF:Ag in the canine serum is 10% of that in plasma. The rationale for developing a serum assay is that serum is easier to prepare than plasma, and less expensive to ship. Moreover, it is frequently available from blood samples that have been collected for other purposes.

We developed a sensitive and specific antibody system for our serum ELISA rather quickly. It was

We developed a sensitive and specific antibody system for our serum ELISA. . .

just a matter of adjusting dilutions to optimize the assay conditions. In addition, we determined that serum vWF antigen correlated with plasma antigen when serum was prepared at room temperature or 37°C. In contrast, vWF antigen levels measured in serum prepared at 5°C did not correlate with plasma vWF antigen. Therefore, if this assay is further developed for diagnostic use, clinicians should not prepare serum in the refrigerator. Finally, we conducted an experiment wherein plasma was drawn and serum prepared from several dogs at the same time. We then measured serum and plasma vWF:Ag and found a good correlation between the two values (Rvalue=0.93). Before this test can be used to diagnose vWD, similar observations must be made on a larger population of dogs.

I would like to thank the NIH and the Richard King Mellon Foundation for sponsoring me in the program this year. I would also like to thank all of the CHL people who were incredibly helpful, including Drs. Catalfamo and Brooks, Mary Spellane, Michelle Brooks, and Fergus.



Leadership was the topic of several meetings.

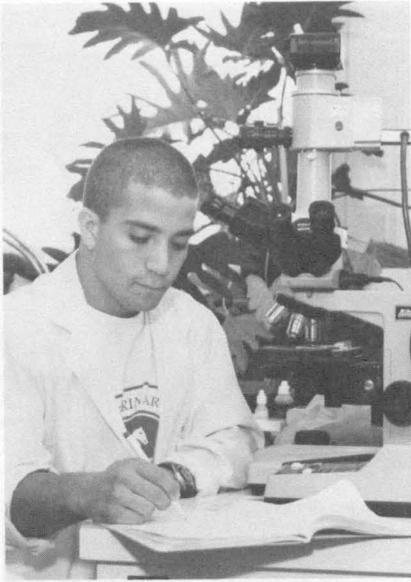


Caroline Murray, Laura Leasburg and Kellie Stephenson at the "Career Day" discussion.



Demian Dressler, Andrew Moorhead, Eileen Adamo and Derek Wilson exchange thoughts on career planning.

Ross Koenig - Cornell University



This summer, I joined Dr. Cornelia Farnum's laboratory as a NIH/Merck Foundation Fellow. My project involved an investigation into the cause of death of terminal hypertrophic chondrocytes during endochondral ossification.

Apoptosis is the physiological mechanism for eliminating unwanted or damaged cells. It is an active form of cell suicide, with individual cells genetically regulating their own demise by *de novo* RNA and protein synthesis. Apoptosis is defined by the specific progression through distinctive morphological and biochemical changes. These changes are quite different from the stages of pathologic cell death, a necrosis in which membrane dysfunction leads to uncontrolled swelling and rupture of cells. In contrast, the apoptotic cell condenses. As the cytoplasm shrinks, the plasma membrane undulates and blebs. The nucleus becomes pyknotic as the chromatin condenses and fragments, while activated endonucleases cleave the DNA at internucleosomal sites. This degradation results in 180-200 base pair lengths, the basis for the hallmark DNA "ladder." Apoptosis occurs

rapidly, over a matter of 30-45 minutes.

A wide variety of stimuli are known to initiate apoptosis. The process has a role in normal embryological development, hormone dependent atrophy, and acts as the natural counterpart to mitosis in self-renewing tissues like skin and intestinal epithelium. The growth plate is an excellent example of a homeostatic self-renewing tissue, with a spatial orientation of the maturing cells providing a temporal sequence of differentiation, hence it is a fine model for studies of both cell proliferation and death, and regulation of these processes.

The fate of the last chondrocytes separated from the metaphyseal vasculature is unknown. In 1987, Dr. Farnum suggested that terminal hypertrophic chondrocytes die by apoptosis. Her hypothesis accords with the characteristic morphological changes in the dying cells and the rapidity of their elimination. Recently, Dr. Farnum identified fragmented DNA in the cells, using the nucleotide end labeling technique. My project this summer was to investigate gene expression of apoptosis-related genes, lending further support to the notion that chondrocytes do, in fact, die by apoptosis. Using immunocytochemical probes, I looked for two different proteins, p21 and bcl-2.

p21 is a cyclin-dependent kinase inhibitor that contributes to cell cycle exit and terminal differentiation leading to cell death. Induction of p21 has also been observed in the differentiation of intestinal epithelial cells *in vivo* and keratinocytes *in vitro*, other cell types believed to die by apoptosis.

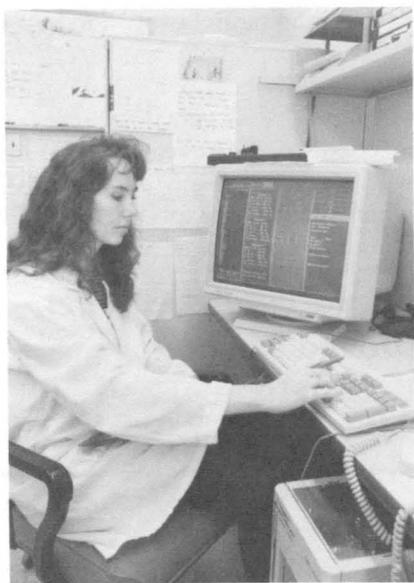
The bcl-2 gene was first identified by its chromosomal translocation in the majority of non-Hodgkins' B cell lymphomas. It is an integral membrane protein found primarily in the nuclear envelope,

endoplasmic reticulum, and outer mitochondrial membrane. By 1990, it was well established that bcl-2 promotes cell survival, blocking PCD. The mechanism is still unknown, but it is thought to involve anti-oxidant protection of the cell membrane.

I used rabbit polyclonal "primary antibodies" to probe for the p21 and bcl-2 proteins in plastic embedded fixed tissue sections. In each case, a biotinylated "secondary antibody," which bound to the primary antibody, was complexed with a streptavidin-peroxidase enzyme conjugate. The latter subsequently reacted with the AEC colorizer to create the positive reaction red colored precipitate. The p21 was localized solely in the hypertrophic zone, supporting the hypothesis that the loss of terminal hypertrophic chondrocytes at the chondroosseous junction is caused by apoptosis. The bcl-2 staining results were ambiguous; therefore we opted to try cryostat sectioning. The program ended as the first results of these tests were obtained, and further analysis is needed before conclusions can be drawn.

The p21 was localized solely in the hypertrophic zone, supporting the hypothesis that the loss of terminal hypertrophic chondrocytes at the chondroosseous junction is caused by apoptosis.

I am extremely grateful to Dr. Farnum, Dr. MacLeod, and Dr. McGregor for guiding me through this fantastic introduction to research.



I have been determined to pursue a career in veterinary medicine since I was very young, but often wondered about the best way to combine this goal with my strong desire to teach. I also developed an interest in research while working on an undergraduate honors thesis in behavioral primatology during my senior year at Cornell University. The Leadership Program provided an opportunity for me to integrate my interests and to learn from other students and faculty members with similar goals.

I enrolled in the program after completing my first year at the Virginia-Maryland Regional College of Veterinary Medicine, and worked in the cardiology laboratory of Dr. Sydney Moise. Todd Nizialek, another leadership student, and I concentrated on two projects concerning a colony of German Shepherds. These dogs provide a model for ventricular arrhythmia and sudden cardiac death. My project involved an assessment of the patterns of ventricular tachycardia (VT) over a period of days. Dogs were monitored for 96 hours with a continuous electrocardiogram.

Using information from previous studies of this colony, Dr. Moise had determined that many of the

subjects die suddenly, often during sleep or quiet periods, and frequently between the ages of four and eight months. After analyzing many 24 hour ambulatory electrocardiographic recordings, researchers in the cardiology laboratory found that the ectopy these dogs experience is not distributed uniformly throughout the day, but tends to display definite peaks. In addition, after developing a system for categorizing behavior, Dr. Moise found that REM sleep and periods of quiet lying were correlated with the most VT.

This background led to three questions:

- 1) Is VT following daily patterns, or is there a pattern distributed over a longer time frame?
- 2) Is the observed 24 hour pattern due to behavior or is it influenced by other factors?
- 3) If the observed pattern is due to behavior, is the crucial factor related to changes in heart rate?

In order to answer these questions, electrocardiographic recordings were made in thirteen dogs, each for a period of 96 hours. The dogs were selected on the basis of previous displays of VT, defined as a run of four or more premature ventricular contractions. One dog with frequent VT was selected to analyze. This dog had died suddenly, but was resuscitated. In this dog, the amount of VT over the course of 96 hours showed peaks, but the peaks were distributed over each of the four 24 hour periods in a manner similar to that observed in earlier 24 hour recordings.

One 24 hour tape was selected from the same dog before and after atrio-ventricular (AV) node ablation, and VT was again counted and correlated with behavior. AV node ablation reduces the variability in heart rate. Although preliminary results suggest that observed rhythms in VT are due in part to behavior and heart rate, other as yet

unknown factors are also crucial.

...preliminary results suggest that observed rhythms in VT are due in part to behavior and heart rate...

One of the most interesting aspects of the research has been Dr. Moise's approach. We began with the history of clinical findings, developed a hypothesis, and tested it using electrocardiographic recordings. Yet in order to fully explain our findings, Dr. Moise incorporated ongoing research into incomplete cardiac sympathetic innervation in these dogs. We met frequently with collaborating researchers and learned that cellular studies of Purkinje fibers from areas lacking proper innervation show prolonged action potential durations, possibly due to altered rate-dependent potassium channels. Our focus thus moved from the whole animal to the molecular level, and then back again. I believe that this method of conducting research and training students is particularly effective.

Although research was the center of the Leadership Program, other aspects were just as enjoyable. I particularly liked interacting with the other students and mentors, and learning about veterinary medicine in other countries and cultures. I would like to thank the NIH and the Merck Foundation for making my experiences this summer possible; Dr. McGregor, Linda Griswold, and Joshua Weisberg for their support of all of us, and most of all Dr. Moise and the rest of the cardiology crew for their guidance and help, and for ensuring that this summer was always fun!

Sally McQuarrie - Massey University



My first encounter with the Veterinary profession was taking my puppy to the Vet for its first vaccinations when I was seven years old. From then on I was completely hooked. I spent summer holidays driving around the West Coast of the South Island with my local vets doing the "James Herriot thing," many happy sunburned days on my Uncle's farm, and endless hours harassing my parents to buy me a horse. A donkey eventuated!!! She was the perfect substitute.

I entered Massey University in 1991 when I was 17 years old, and expect to graduate with the Bachelor of Veterinary Science degree in December 1995. I am interested in all aspects of the profession, but in considering a career in large animals, horses, or surgery at this stage. I had not been involved in research before and this program provided an excellent opportunity to "give it a go." The Dean of my Veterinary School, Professor Stockdale, was extremely supportive of someone from Massey attending the Leadership Program, as no one had ever done so before. So I applied, never expecting to be accepted. It was a pleasant surprise to find I was wrong.

My research was conducted in Dr. Barry Ball's Equine Theriogenology laboratory, with help from Dr. Barry Ball, Dr. Ina Dobrinski, Molly Fagnan, and Todd Skoglund. The objective was to develop the cryostat oviduct tissue model as a method of assessing the molecular basis of spermatozoa adhesion of oviduct epithelial cells (OEC) in the mare. I then used this model to inhibit binding of the spermatozoa to OEC in order to determine the mechanism of adhesion. We were interested in studying this interaction because little is known about the role it may play in longevity of sperm, capacitation, and ability to fertilize the egg.

The mare is unique in that following mating an isthmic sperm reservoir forms in which sperm can remain viable up to four days, whereas in vitro they only live 24 hours. The sperm attach to the epithelium of the isthmus by a lectin-like mechanism, i.e. glycoproteins on the OEC membrane are recognized by the receptors on the sperm head membrane. Lectins are proteins that were originally found in plants. They bind to carbohydrates. It is now known that there are lectins in animals too. Lectins are used extensively to study the surface glycoproteins of cells.

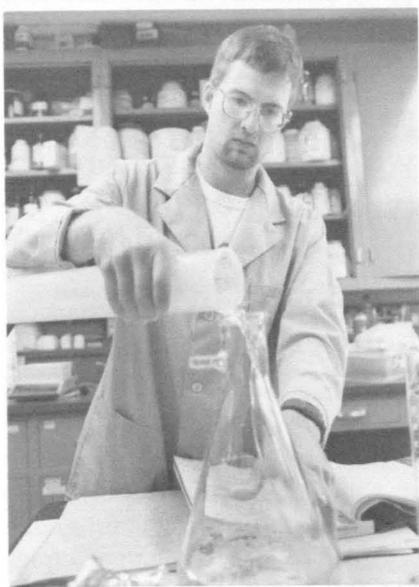
On the theory that a galactose carbohydrate residue on the OEC glycoprotein is responsible for spermatozoa binding, I attempted to inhibit this with asialofetuin (48kD) and galactose. Both bind competitively to receptors on the sperm membrane. I collected semen daily from stallions at the Equine Research Park and incubated it with solutions of fetuin, asialofetuin, galactose, glucose and TALP(control). The solutions were then applied to cryostat sections and sperm attached to the OEC were counted and the OEC area measured, using the NIH Image Macintosh program. Overall

the cryostat model proved to be an effective method of assessing sperm binding, but it could not be conclusively said that galactose was the mechanism. An increase in binding was also seen with asialofetuin, which was not as expected, and still remains to be explained.

...the cryostat model proved to be an effective method of assessing sperm binding...

I have thoroughly enjoyed the Cornell Experience. Cornell University provides an excellent environment for research with many collaborative studies possible. Veterinarians make excellent linkage people, by understanding the scientific data, and having the clinical knowledge to know how to apply this to practical situations. I am extremely grateful to Dr. Ball, Dr. Dobrinski, Molly, Todd; my sponsor, The Richard King Mellon Foundation; and everybody I have met here during my 10 week stay, for making this an excellent learning experience. I would recommend it to anyone who has always considered they would never do research, as you really need to experience it before deciding against such an interesting career path. I hope to one day return to the USA and continue learning, in a residency position preferably, or maybe in a research capacity. Thank you again to everyone I have met for your hospitality, and I extend a welcome to you all to visit New Zealand in the future.

Andrew Moorhead - North Carolina State University



I am currently entering my third year in the veterinary school at North Carolina State University. This summer is my last free summer before I start clinical rotations. I spent it working as a Merck Foundation Fellow in Dr. Judy Appleton's lab at the James A. Baker Institute. Dr. Appleton is an immunoparasitologist whose research interest is in mucosal immunity. She studies the parasitic nematode *Trichinella spiralis*. My research project involved antigen release *in vitro* by *Trichinella spiralis* muscle larvae.

Trichinella spiralis muscle larvae encyst in a host and secrete proteins known as excretory/secretory (ES) antigens. The ES antigens help the larva maintain itself in the muscle cell, which at this stage is known as a "nurse cell." When meat containing nurse cells is consumed, the larvae are activated by the digestive action of the stomach. Released larvae then mature to adults and produce newborn larvae. The newborn larvae migrate through the gut epithelium into the blood and lymph. From there, the larvae migrate to striated muscle and encyst.

I harvested muscle larvae that were encysted in rats. The larvae

were activated by digesting the muscle in a pepsin and hydrochloric acid solution. The worms were isolated from the solution and plated in sterile media in a 96 well micro titer plate (approximately 100-200 muscle larvae per well). Fluid samples from the wells were analyzed for ES antigens via gel electrophoresis and Western Blot. This was the general scheme of every experiment I performed.

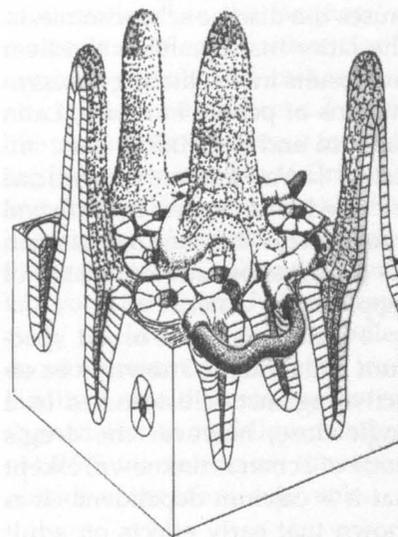
My first task was to examine the amount of antigen released during 24 hours of culture. Muscle larvae release more antigen with time; the longer the incubation time, the more antigen is released. The next step was to stimulate the muscle larvae with various compounds in order to enhance antigen release. One set of compounds was designed to mimic the signal transduction pathway of the worm secretory cells so that secretion would be increased. Diacylglycerol (DAG) and inositol triphosphate (IP3) are both components of the signaling pathway. The muscle larvae were incubated both separately and together with a phorbol ester and ionomycin, which mimic DAG and IP3 respectively. Neither compound produced any noticeable increase in antigen release.

When the muscle larvae were incubated with the wash solution from rat small intestines, secretion was enhanced. In addition, different proteins were evident when the products were analyzed by Western blot. The new pattern was not due to nonspecific binding with gut content proteins, but rather due to a cleavage of the ES antigen by the proteolytic enzymes of the gut contents. This was elucidated by an experiment in which protease inhibitors, gut contents and ES antigen were all combined. The protease inhibitors prevented formation of this new product. Furthermore, when muscle larvae were ex-

posed to gut contents and then washed, they produced unmodified ES antigens. Therefore, it would appear that the muscle larvae produce the new banding pattern in more antigen in the presence of gut contents, but that this antigen is cleaved by intestinal proteases.

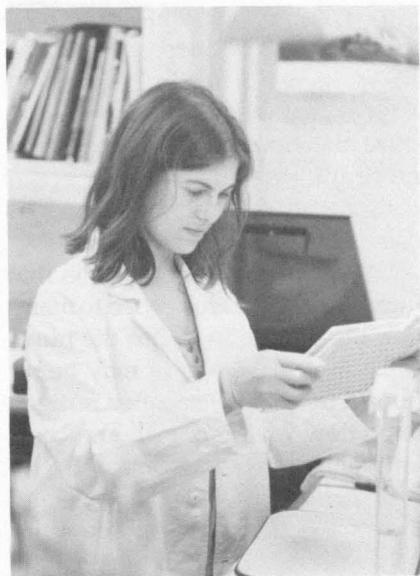
A plausible hypothesis is that intact ES antigens are needed to maintain the muscle larvae in the nurse cell. A cleavage event may be required to alter the secreted antigen and allow invasion of the mucosal epithelium when the larvae are released in the intestine.

Working for Dr. Appleton has been an invaluable experience and I would like to thank her, and the entire lab for their help and support. I would also like to thank Dr. McGregor for making this program possible. Lastly, I would like to thank the NIH and the Merck Foundation for providing me the means to work at The Baker Institute. It has been a most enlightening experience.



Trichinella spiralis larvae migrating through the epithelium of the small intestine

Caroline Murray - The University of Liverpool



Having just completed my intercalating degree of zoology, I was eager to discover further research opportunities available for veterinary graduates. The Cornell Leadership Program provided the ideal opportunity to realize this objective, as well as to develop my academic and social skills. The Mellon Foundation Fellowship I received this summer gave me the opportunity to work with Dr. Edward Pearce in the Department of Microbiology and Immunology, studying *Schistosoma mansoni*, a trematode parasite which causes the disease schistosomiasis. The latter mainly affects the liver and results in debilitating illness in millions of people in Africa, Latin America and the Middle East. Infective larvae known as cercariae are shed into water by infected aquatic snails. Infection occurs when the cercariae penetrate the skin of people in the water.

Praziquantel is a broad spectrum anthelmintic known to be effective against schistosomes in a single dose; however the drug's mode of action is unknown, except that it is calcium dependent. It is known that early effects on adult schistosomes include tetany, vacuolization, surface blebbing and

disintegration of the schistosome tegument, which is at least partly dependent on host immune response. My objective was to ascertain if this mechanism involved changes in surface tegument proteins, resulting in increased exposure of parasite antigens capable of acting as targets for host antibody on the worm surface. In addition I investigated the extent of surface protein phosphorylation in praziquantel-treated worms.

The schistosome surface consists of a lipid bilayer. In order to specifically examine surface proteins, it was necessary to distinguish them from nonsurface proteins. This was achieved by biotinylation of the tegument; biotin (as utilized) binds covalently to surface proteins only, which can then be identified using Streptavidin, a protein which binds specifically to biotin. In practice, the biotinylated proteins were extracted from the parasites with detergent, and electrophoresed on polyacrylamide gel, which separates proteins according to their molecular weight. They were then transferred to a membrane, and detected with labelled streptavidin. Using this technique, I was able to demonstrate that praziquantel treatment of worms both in vivo and in vitro leads to the exposure at the parasite surface of normally sequestered proteins.

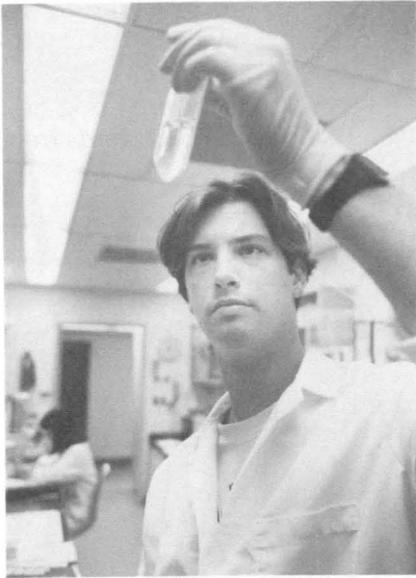
In order to identify kinases exposed at the surface membrane of schistosomes, I performed in vitro kinase assays with Streptavidin-agarose-precipitated biotinylated surface proteins. Using this technique, and supported by evidence obtained from Western blots of surface proteins using a monoclonal antibody specific for phosphotyrosine, I was able to demonstrate that praziquantel-treated worms express more tyrosine-phosphorylated surface proteins than do nontreated worms.

...I was able to demonstrate that praziquantel-treated worms express more tyrosine-phosphorylated surface proteins than do nontreated worms.

The Cornell Leadership Program was a unique and very rewarding experience. It allowed me to actively participate in cutting edge molecular biology in an encouraging and stimulating environment. It also enabled me to meet a diverse range of multicultural students, of which I will always have many fond memories.

I would like to express my enormous thanks to my work colleagues who continually provided support and advice throughout my stay, and to whom I am eternally grateful: Dr. Edward Pearce, Esther Racoosin, Stephen Davies, Beth Sabin, Joao Pedras and Susan Leonard. I look forward to enhancing my newly learned skills.

Anthony Mutsaers - Ontario Veterinary College



My interest in research began courtesy of John Labatt Ltd., where I spent two semesters as a research assistant while I was an undergraduate student in the co-operative biology program at the University of Waterloo. The summer preceding my entry into the Ontario Veterinary College was spent at Agriculture Canada's Animal Diseases Research Institute, where I obtained my first exposure to veterinarians engaged in research. Last summer I had the opportunity to work with Dr. Mark Hurtig in the department of clinical studies at the OVC, where I was involved in the study of non-invasive methods for the diagnosis of equine developmental orthopedic diseases. Conversations with various Leadership Program alumni convinced me to apply to the program in order to further develop my research skills and broaden my knowledge of opportunities in research-based veterinary medicine.

As a Mellon Foundation Fellow, I had the opportunity to work with Professor Jun-Lin Guan, and postdoctoral fellow Noriaki Imanishi, in the Cancer Biology Laboratories of the Department of Pathology. Dr. Guan's lab is exploring the functions of the cyto-

plasmic protein tyrosine kinase pp125^{FAK} (focal adhesion kinase, FAK) in signal transduction by integrin cell surface receptors. FAK is composed of a centrally located kinase domain (K) flanked by N and C terminal regions. My project dealt with the identification of proteins which interact with the NK region of FAK, using a yeast two-hybrid system.

In this two-hybrid system, interacting proteins can be identified by assaying yeast cells for their growth on nutrient (histidine) deficient agar, coupled with the production of β -galactosidase which cleaves X-gal substrate yielding a blue color.

Colonies were screened for growth ability and β -galactosidase activity, yielding twenty four double-positive clones which were subsequently characterized. After retransformation of these clones into HF7c yeast cells and retesting for growth and β -galactosidase activity, one clone produced very promising results. My project entailed the incorporation of this 1.5kb cDNA clone, named NK-interacting protein #17 (NKIP17), into various plasmid vectors for further study of the protein itself, as well as its interaction with FAK.

I began by generating a pGBT9-nkip17 construct. This recombinant plasmid will be used in the future to study the binding ability of NKIP17 to FAK as well as a variety of mutated FAK proteins, in an attempt to isolate the exact region of FAK where binding occurs. I also successfully ligated NKIP17 to the pGEX 1 vector. This vector contains an open reading frame encoding glutathione-S-transferase (GST). GST-fusion proteins expressed when induced with isopropyl-1-thio- β -D-galactoside (IPTG). The protein can then be purified by affinity chromatography. Antibodies to NKIP17 can then be prepared that will be

helpful in further studies of FAK-NKIP17 binding using techniques such as Western blotting. The GST-fusion protein obtained from the specified vector was found to be very insoluble. An attempt was made to improve its solubility. To this end, I prepared two new pGEX recombinant plasmids incorporating NKIP17 cDNA which had been truncated at the 3' end (0.2kb and 0.8kb out of the 1.5kb cDNA fragment). Finally, I was able to successfully ligate NKIP17 to the pKH3 plasmid vector. The pKH3 vector will allow expression of NKIP17 in mammalian cell lines such as cos cells. In addition, it contains three repeats of an influenza virus hemagglutinin epitope, which will allow for identification of NKIP17 expression in cos cells using a monoclonal antibody against this epitope.

...I was able to successfully ligate NKIP17 to the pKH3 plasmid vector.

I have thoroughly enjoyed my stay at Cornell. The many experiences that the Leadership Program offers have allowed me to become conversational in the language of molecular biology, further clarify my career goals and make many new friends in the process. I would like to thank Dr. McGregor for making it possible and Linda Griswold and Joshua Weisberg for all their organizational efforts. I would also like to thank Dr. Guan and his entire lab group for all their help and especially Dr. Imanishi for his endless encouragement, patience and trust. Finally, I would like to express my appreciation to my fellow members of LP '95 for making my time spent outside of the lab so enjoyable.

Todd Nizialek - University of Georgia



I am a rising junior at the University of Georgia College of Veterinary Medicine. After attending Duke University on a Naval ROTC scholarship and graduating with a B.S. in Zoology in 1989, I spent four years as a Supply Officer in the U.S. Navy. Although this experience was rewarding, I resigned my commission to pursue a career in veterinary medicine.

I saw in the Leadership Program an opportunity to become involved in research and appreciate how research forms the basis of clinical medicine. Together with fellow Leadership student Laura Leasburg, I joined Dr. N. Sydney Moise's research team. Dr. Moise has been studying cardiac arrhythmias in a colony of German Shepherd Dogs. Although Laura and I became involved in several projects, I will discuss only one of them - the Sudden Cardiac Death Study.

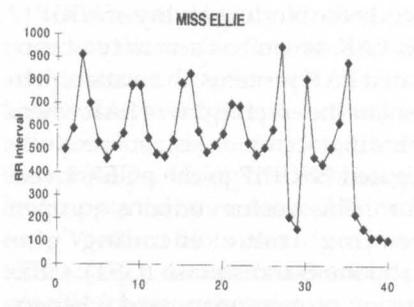
We studied thirteen dogs that died suddenly. All had documented histories of frequent ectopic ventricular beats, manifested as premature ventricular contractions (PVCs) or ventricular tachycardia (VT=runs of 4 or more PVCs). Previous studies had shown that dogs with frequent VT more often die than dogs

with infrequent VT. Although the dogs' cardiac activity immediately prior to death was not recorded, it is assumed that VT leads to ventricular fibrillation and sudden death. Therefore, we wanted to study the most severe VT in the subject dogs.

A pressing question was whether a specific surface ECG pattern precedes the onset of VT. The question has important clinical implications, but identification of a pathogenic ECG pattern as a predictor of VT has the additional prospect of better understanding the mechanism of this arrhythmia. Three factors must be considered; the heart tissue, modulating factors, and triggering factors. Our study addressed the latter.

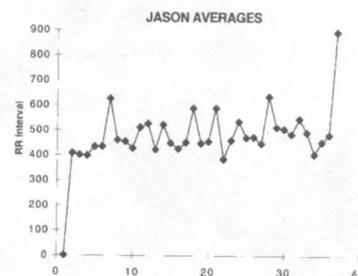
From 24-hour electrocardiographic monitoring tapes, we selected the ten longest runs of VT between midnight and 8:00 A.M. on each dog closest to the time of the animal's death. We did so because previous studies had shown a higher level of ectopy in association with lying/REM sleep. After identifying the runs, we measured R-R intervals (the time between each beat) for the 20 seconds prior to a run.

After graphing the R-R intervals for each run in individual dogs, we searched for a characteristic pattern. Similarity was observed in the runs from one dog ("Miss Ellie"),



but the same pattern was not observed in other dogs. However, we did note a relatively long R-R interval - not necessarily the longest of

the 20 seconds - just prior to the ventricular tachycardia. We averaged and graphed the R-R intervals of the runs within each dog (see "Jason"), moving backwards from



the episode of VT. In every dog, we found the average R-R interval just prior to the VT was the longest. Evidently, this pause or long R-R interval is related to the onset of VT.

Our findings accord with previous studies of dogs from the same colony in which phenylephrine administration or AV nodal ablation had lowered the ventricular rate, lengthened the R-R interval, and increased the severity of the arrhythmias. This was similar to the pause prior to the VT that we observed in our study. While the observed pattern may not be unique to the VT of the dogs that died suddenly, further work can be done to determine whether the pattern preceding the VT of living affected dogs is any different.

In closing, I would like to offer my sincere thanks to the NIH and the Robert W. Woodruff Foundation for sponsoring me this summer, and to Dr. McGregor, Linda Griswold, and Joshua Weisberg for the time and energy which they devoted to every detail of the program. I would also like to thank Dr. Moise for serving as my mentor, and the "kids" from the "Cardiology Crew" for their patience, understanding, and friendship.

Kellie Stephenson - University of Sydney



I am currently in my fifth and final year of the veterinary science program at the University of Sydney. In 1993, I interrupted my veterinary training to complete the requirements for a research degree, Bachelor of Science (Veterinary). The research aspect of my training, unlike the present, was more applied than basic. I applied to this program, not only to see a new country, but to experience a different aspect of research in new cultural surroundings.

Meeting new people and sharing cultures has been a wonderful experience. Since we have a common goal of veterinary science and are wanting to get the most out of life, we blended very well. I made lifelong friendships.

My research has involved investigating recombinant canine erythropoietin (rcEPO). Ten weeks is a very short time in which to accomplish much in research, and thus it has been frustrating for me not to be able to involve myself more in the project despite my best effort. Nevertheless, I participated in several aspects of the project that spanned molecular biology—Western blots and Northern blots—tissue culture work and bioactivity

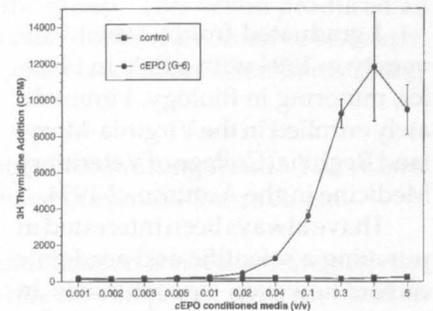
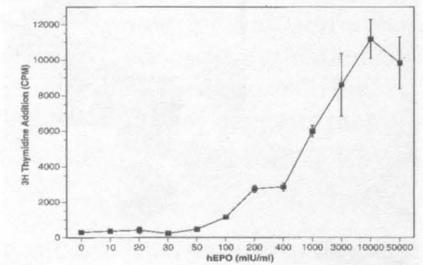
studies. I will discuss the bioactivity experiments, for this aspect of the project alone was completed in the brief time that I was here.

Chronic renal failure is common in many species. The pathogenesis involves non-discriminant destruction of renal cells. Erythropoietin is normally produced by peritubular cells of the kidney. When the kidney is hypoxic, the production of erythropoietin is enhanced. This protein is then transported via the blood to the bone marrow, where it stimulates haematopoiesis. In chronic renal failure, erythropoietin production drops and often becomes inadequate to support the requirements for normal red blood cell replacement. This results in the development of anemia. Many of the clinical signs of chronic renal failure can actually be attributed to this anemia. Therefore, if animals with this problem are given exogenous erythropoietin as a therapeutic supplement, their anemia can be corrected and their quality of life improved.

Prior to my arrival, the canine erythropoietin (cEPO) gene had been cloned and produced in my mentor's laboratory. My task was to ascertain whether the protein was biologically "active" - i.e. whether it functioned as expected by enhancing erythropoiesis. The bioactivity studies using rcEPO were performed both in vitro and in vivo.

IN VITRO: Erythroid precursor cells, including proerythroblasts and erythroblasts were harvested from the spleens of mice and cultured in medium containing predetermined concentrations of rcEPO or a commercially available preparation of human recombinant erythropoietin (rhEPO). The rhEPO was used to generate a standard curve in order to ascertain the relative potency of its canine counterpart. To this end, cellular proliferation was measured by ^3H -thymidine incorporation into

newly synthesized DNA. Incorporation of the radionuclide was quantified in a liquid scintillation counter. The results are shown in Figures 1 (rhEPO) and 2 (rcEPO) and demonstrate that both proteins strongly stimulate replication of the erythroid precursor cells.



IN-VIVO: Mice were injected subcutaneously with Phosphate Buffered Saline (control), rhEPO (20 IU/ mouse) or rcEPO (8.5 IU/ mouse) on three consecutive days. Blood was collected on the fourth day and the number of newly formed erythrocytes (reticulocytes) were quantified by flow cytometry. The reticulocyte counts, expressed as a percent of total RBC were 3.2 ± 0.8 , 11.5 ± 2.0 and 6.4 ± 1.0 for the control, rhEPO and rcEPO recipients, respectively. Again, these data demonstrate that rcEPO is biologically active and stimulates red blood cell production.

I would like to thank the Woodruff Foundation for providing me with a fellowship. Thank you Jamie MacLeod, my mentor, Jon Tetreault, Jane Miller, and Da Nian Gu for your guidance, assistance and an unforgettable experience.

Derek Wilson - Virginia-Maryland Regional College of Veterinary Medicine



I graduated from Cornell University in 1994 with a B.A. in Physics, minoring in Biology. I immediately enrolled in the Virginia-Maryland Regional College of Veterinary Medicine in the Autumn of 1994.

I have always been interested in pursuing a scientific and academic career; however, opportunities in biology are few and far between for students whose background is in physics. Never having participated in basic research, I had little idea what to expect from my experiences in the Leadership Program.

As a Merck Foundation Fellow, and with additional support from the NIH, I worked in Dr. Jharna Ray's laboratory at the James A. Baker Institute for Animal Health. The focus of Dr. Ray's studies are lysosomal storage diseases in humans and animals. Having perfected a technique for harvesting and growing the retinal pigmented epithelial cells (RPE) from the eye, she has made great progress towards establishing animal models for the diseases.

Lysosomal storage diseases are a heterogeneous group of genetic disorders that are expressed as deficiencies in lysosomal enzymes important for the normal breakdown

of cellular and extracellular matrix components. Disease ensues when undegraded substrates of the deficient enzymes accumulate causing cellular swelling and deformation, disrupting the proper function of the affected cell, as well as neighboring cells. People and animals with these diseases suffer from skeletal abnormalities, mental retardation, hepatosplenomegaly, corneal clouding, and ultimately early death.

β -glucuronidase (GUSB) is an important lysosomal enzyme that is responsible for the breakdown of glycosaminoglycans (GAGs), a major component of tissue matrices. In the eye, the retinal pigment epithelium is surrounded by an extracellular matrix, which is constantly turning over. Abnormalities in matrix turnover result in visual cell disease and degeneration leading to blindness. GUSB deficiency is the cause of Mucopolysaccharidosis VII (MPS VII), a disease identified in humans, canines, mice and felines. The sequence of the cDNA and therefore the protein has been completely or partially determined in all but the feline. While there has been limited success in establishing a breeding colony of MPS VII affected cats, the unusually high expression of β -glucuronidase in feline RPE makes the cat an especially important model.

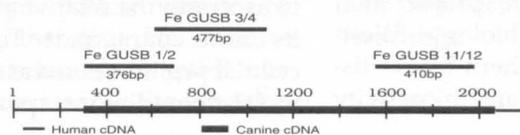
Using reverse transcriptase-polymerase chain reaction (RT-PCR), I attempted to isolate three regions of the normal, wild-type feline cDNA from feline RNA provided by Dr. Ray's lab. I was excited to successfully amplify all three expected size segments of feline cDNA using canine-specific primers. Properly optimized RT-PCR reactions allowed me to isolate the fragments

sufficiently clean to ligate them into a plasmid vector.

I transformed *E. coli* with the ligation mixture and analyzed several bacterial clones. Clones containing GUSB cDNA fragments were selected and one of them was used to prepare large amounts of pure plasmid for sequencing.

Results of sequencing were very encouraging. There was over 80% identity between my feline GUSB fragments and the published sequence of human GUSB cDNA. The greatest similarity in the translated protein sequence was found to be in the region of the substrate binding site, which was almost entirely within the sequenced feline GUSB segment. Determination of the remainder of the coding sequence will take only a few more weeks when the work is continued. Once we characterize the normal cDNA sequence, we may begin looking for mutation in the cDNA which results in the defective enzyme.

I would like to express my great appreciation to Dr. Jharna Ray for her advice, patience, understanding, and especially her accommodating sense of humor throughout the summer. Thanks, too, to Dr. Gus Aguirre for the fine food and endless stories. Additional thanks to Dr. Kunal Ray and Dr. Greg Acland for much needed and appreciated advice and encouragement. I would also like to thank Linda Griswold for her tireless cheer in managing the unmanageable and performing the impossible. Special thanks to Dr. McGregor for his vision and dedication to create this program and especially for bringing together the most wonderful and exciting group of people I have met. To all my new friends with whom I've spent the Summer, THANK YOU!



Ethics Committee Report

Demian Dressler, Gertraut Altreuther and Elizabeth Adkins

We discussed four scenarios from the Science World Wide Web site. The first scenario addressed the question whether a project design and data belong to the principal investigator or the graduate student who performed the work. A possible solution to this issue would be for the two individuals to publish the results jointly. This would be advantageous because it would maintain a good relationship. One cannot predict when these individuals might wish to work together again. Most of those who took part in the discussion felt that all parties to issues of this sort should strive to maintain friendly relationships.

The second scenario considered the ownership of data under the Freedom of Information Act. Here the discussion focused on a study performed at a government institution that was never published. Another individual who was not directly involved in the investigation retrieved and published the data under the Freedom of Information Act. The group expressed the opinion that the scientist who performed the work should have been informed that his data had been provided to a third party and that it might be published. Common courtesy suggests that the individual requesting the data should have done so himself. The group felt that the individual who generated the data had the right to publish it whenever he or she desired.

The third scenario concerned a student who accused a fellow student of falsifying data. After discussing this with the principal investigator, the accuser felt her concerns were given insufficient attention. At a laboratory meeting the accuser produced photocopies of the falsified data for all members of the group. This action produced an uproar. The outcome might have been avoided had the senior researcher taken the accusation more seriously. The group discussed the issue at length, and concluded that accusers, even when correct, are often unjustifiably criticized by the scientific community.

The fourth scenario concerned a professor who accused one of his students of committing fraud. The reason offered was the student's poor note keeping. Careful notekeeping would have prevented this problem.

The recurring theme in all of these scenarios is poor communication. Communication is not a passive activity; it requires effort by all concerned. The group expressed the thought that while they hope not to be involved in situations of this sort, they gained insight from the discussions.

The second portion of the evening was devoted to a skit, performed by four of the students. It dramatized one of the many ethical issues raised in Carl Djerassi's novel *Cantor's Dilemma*. The skit portrayed a romance between a professor and one of her former students, whose career development benefited from the professor's involvement. After much applause, a lively discussion ensued that focused on the ethical implications of platonic and non-platonic student/teacher relationships. It was resolved that a non-platonic relationship between a student and a teacher is acceptable if the teacher is not in a position to affect the student's career development.

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So many smiles
A thousand petals raining down
In tiny precious fleeting drops
And so much sun
Entwined the dancing hands and feet
That came to learn and share and grow
But so much time
So quickly spent
The freshly shrouded crossing point
Stands heavy, silent soul-less now
Where faint and dying whispers tell
Of what is sweet and what is lost

By: Danielle Greenberg
8/11/95

NOTES:

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Photographs by Dede Hatch Photography.