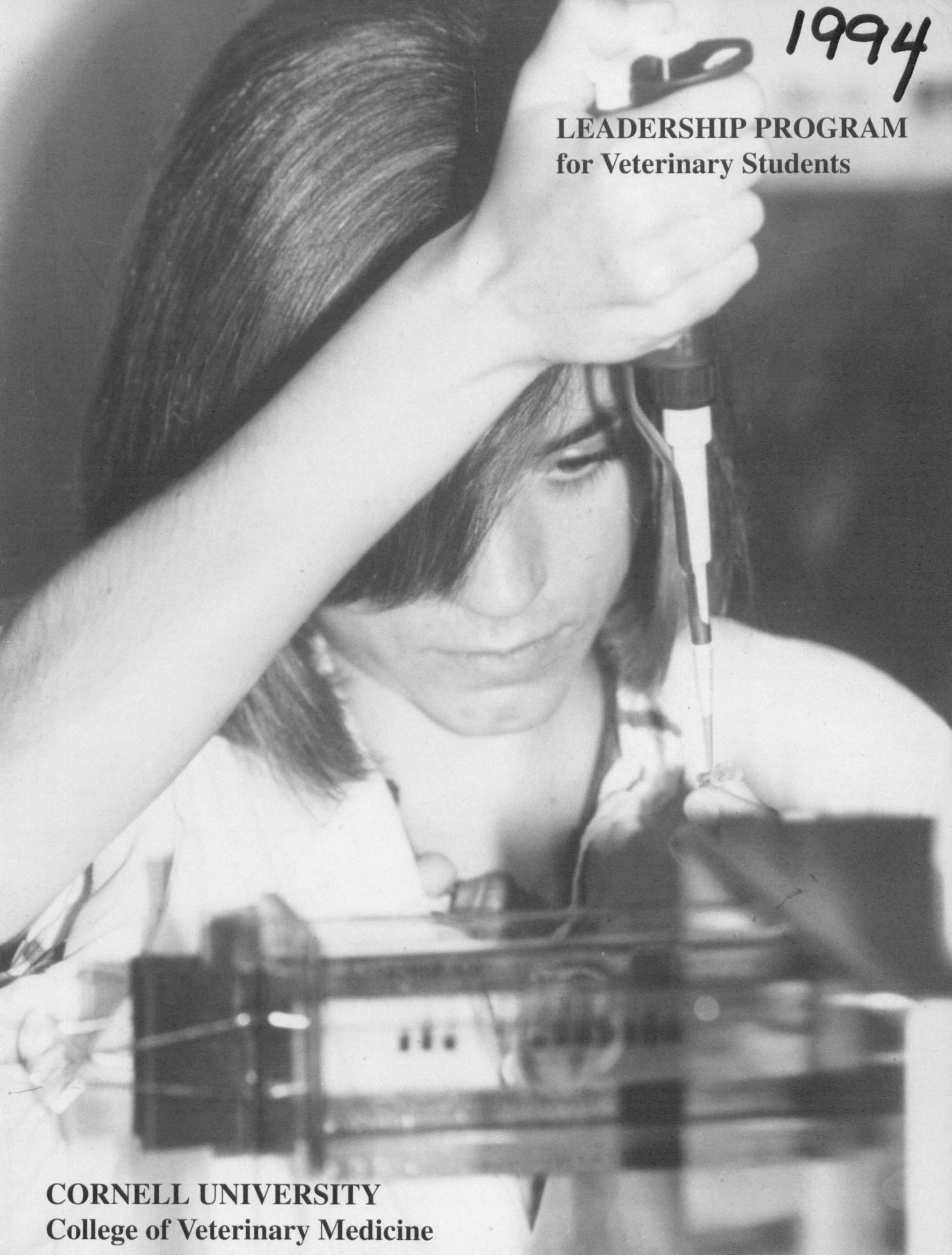
1994

LEADERSHIP PROGRAM
for Veterinary Students



CORNELL UNIVERSITY
College of Veterinary Medicine

Program Overview

Nineteen ninety-four marked the fifth consecutive year in which the College of Veterinary Medicine at Cornell hosted a Leadership Program for Veterinary Students. The program combined faculty-guided research with a variety of professional enrichment activities calculated to enhance the problem solving skills of the students, their leadership ability and commitment to a research career.

"Class of 1994"

Twenty-one students from thirteen different veterinary colleges in the United States, Canada, Australia, the United Kingdom, and Spain took part in the program this year. Carol Miltenberger, a 1994 graduate of the College of Agriculture and Life Sciences at Cornell, served as Program Coordinator. Leslie Gabor (Sydney University) and Gwendolyn Jeun (Ontario Veterinary College) were elected by their fellow students as "Program Representatives." Both worked with the program organizers and with Carol in facilitating events connected with the program.

The program spanned ten weeks during the months of June, July, and August. Participating students were assigned research projects that enabled them to explore a variety of subjects; to learn new investigative techniques, and to gain insight into how research laboratories utilize their professional and material resources. It was a comprehensive learning experience. In addition to research, the students participated in activities intended to heighten their awareness of ethical issues and the meaning of leadership, while simultaneously improving their critical capacity and communication skills. Many of the events were organized by the students, thereby enabling them to actively structure their own education. Salient features of the training experience are described below.

Dr. Jeff Ward addressing the students on "Career Day."

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 individuals who have achieved distinction as research scientists and administrators, or who are in an advanced stage in their own training, served as discussion leaders and counselors. The following individuals took part:

- Dr. Patricia M. Chisholm, Scientific Program Manager, The Wellcome Trust, London, England
- Dr. Robert E. Donahue, Director, Simian Gene Transfer Program, National Heart, Lung and Blood Institute, Clinical Hematology Branch, National Institutes of Health, Washington, DC
- Dr. James N. MacLeod, Assistant Professor of Molecular Genetics, College of Veterinary Medicine, Cornell University
- Dr. Jeffrey L. Ward, Ph.D. Candidate, Graduate Program for Veterinary Clinician Scientists, Cornell University

The students and counselors developed an agenda on the evening preceeding the meeting. The meeting itself included introductory remarks by the students and presentations by the counselors. The latter were followed by breakout sessions in which the students and counselors explored aspects of career planning. That evening, the students entertained their visitors, faculty mentors, representatives of the program's sponsors, and other invited guests at a dinner held in Willard Straight Hall.

At a separate meeting, Professors Brian R.H. Farrow, Francis A. Kallfelz, and Robert M. Lewis led an evening discussion of internship and residency training. Factors that veterinary graduates should weigh in evaluating clinical training programs were discussed. The discussion leaders also explained how some residency training initiatives interface with graduate research. On another occasion, Professors Barbara A. Baird, Richard A. Cerione and Edward J. Pearce convened a meeting in which critical issues relating to research training were discussed.

Dr. Michelle Haven described her research and administrative duties at Merck and Co.

Two scientists from the Research Laboratories of Merck and Co., Dr. Gerard J. Hickey and Dr. Michelle L. Haven, discussed career opportunities for veterinarians in industry. They also described their own research and administrative duties, and the hiring practices of Merck and Co. The meeting was much appreciated by the students, several of whom have expressed an interest in a career in industry.

Leadership Discussion: Many of the events connected with the Leadership Program enabled students to reflect on the responsibilities of leadership. Participants in this year's program were provided with a personal copy of John Gardner's book, *"On Leadership."* Soon after their arrival, Dr. Robert D. Phemister, Dean of the Veterinary College at Cornell, met with the students to organize a meeting to address the issues raised by Gardner. The meeting capitalized on a technique borrowed from training programs based on the "total quality management" concept. The students divided themselves into three groups. Dean Phemister, Dr. David R. Fraser, Dean of the Faculty of Veterinary Science at the University of Sydney, and Dr. Peter M. Biggs, recently retired Director of the United

Deans David Fraser and Robert Phemister setting the stage for the "Leadership Discussion."

Amanda Gaskin discussing aspects of the ethics "project" with Professor Ari van Tienhoven.

Kingdom's Agricultural Research Council, served as moderators. The students identified the characteristics of a leader, attached priorities to these attributes, shared their conclusions with members of the other two groups, and arrived at a consensus.

Biomedical Ethics: Dr. Ari van Tienhoven, Emeritus Professor of Animal Physiology and one of Cornell's finest teachers, organized a "project" in biomedical ethics. During the ten-week period in which the program was in session, the students investigated and reported on a major ethical issue that captured public attention several years ago. The students were provided copies of several key articles and editorials, including the original paper which lay at the center of the controversy. They also received copies of the congressional testimony connected with the case. After several weeks of independent study, the students met to discuss the issues and reached a consensus on how such matters should be managed. Their conclusions are documented in this report. On the day following the discussion, Ms. Sue Baptiste, Assistant Professor, School of Occupational Therapy & Physiotherapy at McMaster University, moderated a discussion of the advantages and limitations of group learning.

Radiation Safety: Radioisotopes are used frequently in research; yet many investigators are unfamiliar with procedures connected with their proper acquisition, use and disposal. Participants in the 1994 program received four hours of instruction on such matters. The presentations were arranged by Cornell's Radiation Safety Officer, Mr. Thomas J. McGiff.

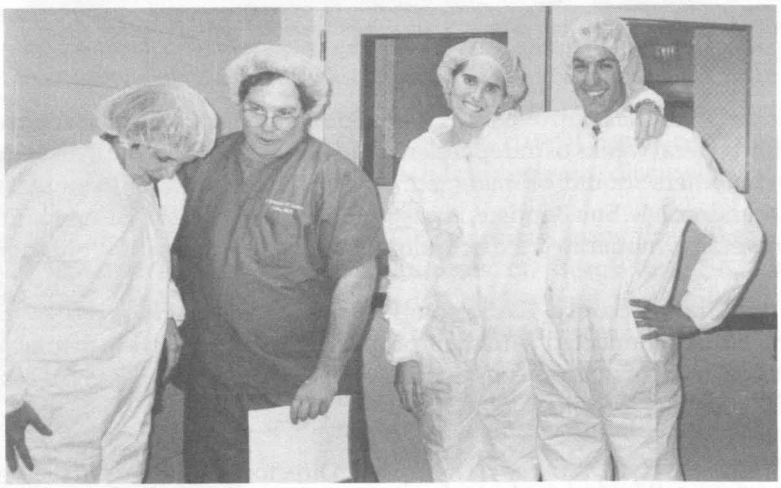
Life Safety: Dr. Larry J. Thompson, Director of Biosafety in the Veterinary College, presided at a meeting that reviewed general principles governing the handling, storage and disposal of hazardous chemicals. Dr. Thompson also reviewed the College's policy governing the protection of employees from work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

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 in the host institutions, and to grasp the scope of research and opportunities for advanced training at the NIH and the USDA. Agendae for the visits are reproduced below.

National Institutes of Health
June 30, 1994
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. Pam Dressell, VRP, Office of Client Relations, "Overview of NIH, NCRR, and VRP"
9:15 a.m.	Dr. Victoria Hampshire, Chief, VRP Carnivore and Ungulate Unit, "The NIH Miniature Pig as a model for gene therapy"
9:30 a.m.	Dr. Michael Eckhaus, Chief, VRP Pathology Unit, "Nephropathy in transgenic mice containing a partial HIV1 genome" "Career opportunities for veterinarians in the U.S. Public Health Service"
10:30 a.m.	Dr. Michael Fordis, NIH Office of Education,

	"NIH Research Fellowship and Training Opportunities"
11:30 a.m.	Dr. LaRoy Penix, NINDS, "New Developments in Epilepsy Research: From Basic Mechanisms to Clinical Applications"
Noon	Lunch Break
1:45 p.m.	Dr. Jefferson Mitchell, NINDS Laboratory of Experimental Neuropathology, "Studies of Infections Caused by Herpes Viruses"
2:30 p.m.	Dr. Clarence J. Gibbs, NINDS Laboratory of Central Nervous System Studies, "Slow Infections of the CNS"
3:30 p.m.	Dr. Robert Donahue, NHLBI Clinical Hematology Branch "Discussion of Gene Therapy Strategies"; Tour of Facilities
5:15 p.m.	Final Review



Mary Thompson and Oliver Turner toured the intensive care unit of the NHLBI Clinical Hematology Branch. Mr. Earl West explained the operation of the facility to Ms. Maureen Mylander who coordinated the visit.

United States Department of Agriculture
(Beltsville Campus)
July 1, 1994

Research Programs of the USDA

8:30 a.m.	Dr. T.J. Sexton, Director, Livestock and Poultry Sciences Institute, "Overview of the Livestock and Poultry Sciences Institute"
9:00 a.m.	Dr. R. Gamble, Acting Research Leader, Parasite Biology and Epidemiology Lab, "Food-borne parasites associated with livestock"
9:30 a.m.	Dr. J. Lunney, Acting Research Leader, Parasite Immunology Laboratory, "Genetics and disease resistance"
10:00 a.m.	Break
10:30 a.m.	Dr. Robert Wall, Research Physiologist, Gene Evaluation and Mapping Laboratory, "Transgenic Animal Research"
Noon	Departure

Professor Robert Gilbert and Sonia Mumford reviewing materials provided by their hosts at the USDA.

Student Presentations: Before leaving Cornell, the students reported on their research activities in a meeting which faculty and administrative staff of the College were invited. The presentations were uniformly good, and some were outstanding.

Program Sponsors: The 1994 Leadership Program was sponsored jointly by The Richard King Mellon Foundation, The Merck Foundation, and The Robert W. Woodruff Foundation. In addition, The Wellcome Trust and La Fundación Purina provided fellowships for students from the United Kingdom and Spain, respectively. The Auxiliary of the New York State Veterinary Medical Society provided each student with a copy of the program text and several book prizes.

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

Mr. Arthur Miltenberger represented the Richard King Mellon Foundation at the Career Day Dinner

Joseph Alaimo - Cornell University

I have not always wanted to be a veterinarian, although I admit that it was a dream throughout much of my childhood. Instead I chose, in my early adulthood, to look at other careers - ones that I thought were easier to obtain or were financially more accessible. It was not until I was 23 years old that I realized that the value gained in a career can be directly proportional to the effort one makes. And so, four under grad and one grad year later, I find myself between my first and second years of vet school at Cornell. My goals are to teach and conduct biomedical research. I was very happy to have been given the opportunity, through the Leadership Program, to pursue research in the area of science that I find most interesting. It was an interesting coincidence that my sponsor, The Robert W. Woodruff Foundation, came into being through the generosity of the individual who built Coca-Cola into the world wide company that it is. Sponsorship by the Woodruff Foundation had special meaning for me because my father was a Coke machine repairman in Brooklyn.

My summer research project was conducted in the laboratory of Dr. James MacLeod at the James A. Baker Institute for Animal Health. It centered on the comparative expression of growth hormone (GH) and insulin like growth factor (IGF-I) in three coisogenic strains of mice. The first strain was a dwarf line, containing a mutation in the gene that encodes hypothalamic GH releasing factor. The mutation suppresses synthesis and release of GH by the pituitary gland. The second strain - the control or "wild type" - expresses normal amounts of GH. The third strain was a transgenic giant. Three extra copies of the GH gene were introduced into this line by oocyte microinjection, which results in significantly elevated levels of circulating GH. The expression of GH and its subsequent effects can be seen through the phenotypic traits associated with each mouse strain. At five weeks of age, the dwarf, wild type, and GH-transgenic mice weigh on average 9, 17, and 21 grams, respectively.

To study the effects of GH on IGF-I expression, total ribonucleic acid (RNA) was extracted from liver tissue and visualized by agarose electrophoresis with ethidium bromide staining. The RNA from the gel was then transferred to a nylon membrane. Radiolabeled cDNA probes for murine IGF-I, rat GH, and rat elongation factor Tu (EFTU) were made. Hybridization of the ^{32}P-labelled probes to the RNA on the membrane was visualized by autoradiography. Hybridization band sizes were determined by correlation to a commercial RNA ladder.

Expression of EFTU is not strain dependent. This probe was used to normalize the amount of RNA between samples. The probe for GH hybridized to the transgenic mouse liver samples, but not the wild type or dwarf samples. This finding is consistent with the fact that in normal mice, GH is expressed only in somatotrope cells in the pituitary gland, while in transgenic mice, expression of the GH transgene is regulated by a metallothionein promoter that permits its transcription in many tissues. Northern blot analysis with the IGF-I probe revealed IGF-I transcription in liver samples of both the wild type and giant mice but not in the dwarf strain. This clearly illustrates the relationship of GH to the expression of hepatic IGF-I. The amount of IGF-I mRNA is dramatically reduced by the absence of circulating GH in the dwarf mice.

In situ hybridization offers the potential to examine gene transcription in individual cells while maintaining normal tissue architecture. To this end, liver tissue from dwarf and normal lines are being fixed, embedded, and cut into 1.8 mm sections. A ^{33}P-labeled riboprobe will then be hybridized to the sections at 45°C overnight, coated with autoradiographic emulsion, and developed after five days exposure. Expression of IGF-I in hepatocytes should parallel patterns seen by Northern blot analysis. I will continue this work in the fall and hope to apply these techniques to chondrocyte differentiation in the proximal tibia growth plate.

"Northern blot analysis revealed IGF-I transcription in liver samples of both the wild type and giant mice but not in the dwarf strain."

Melissa Beall - Ohio State University

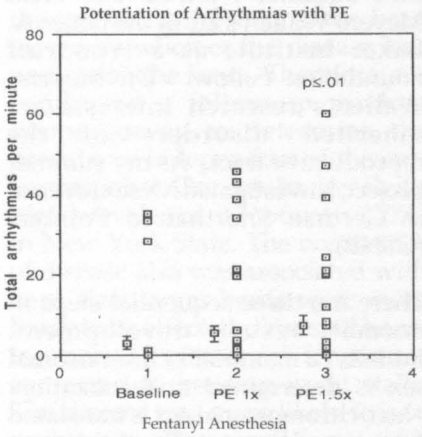
Prior to entering veterinary school in 1991, I completed a Bachelor of Arts degree in Chemistry at Cornell University and a MS degree in Neuroscience at the University of Pittsburgh. My research experience during this time included behavioral studies of learning and memory as well as neuroanatomical studies of the entorhinal cortex and hippocampus. I entered veterinary school in order to obtain a more complete understanding of physiology and disease mechanisms. I plan to continue my clinical and research training after graduation with the hope of one day applying both my veterinary and research skills to the study of animal models of disease.

As a Mellon Foundation Fellow, I was afforded the opportunity to participate in an investigation conducted by Dr. Sydney Moise. Her research focuses on the study of a colony of German Shepherd Dogs with inherited ventricular arrhythmias. The dogs have the tendency to die suddenly early in life, often in their sleep. Dr. Moise and members of her lab have spent the last four years investigating the heritability and cause of the ventricular arrhythmias. Their hypothesis is that the autonomic innervation of the heart develops abnormally in affected dogs. They have demonstrated that the dogs' ventricular arrhythmias develop, on average, at twenty weeks of age, tend to be more prominent during REM sleep and almost always occur within a few beats of a long RR interval. As a result of this work, they have concluded that elevated parasympathetic tone and a slow heart rate may be critical factors associated with the initiation of the ventricular arrhythmias.

In order to test the hypothesis that elevated parasympathetic tone could precipitate the arrhythmias, a pharmacological protocol utilizing phenylephrine (PE) was developed. Phenylephrine, an alpha agonist, was chosen because it produces, through peripheral vasoconstriction and elevated blood pressure, a reflex mediated increase in vagal tone and slowing of the heart rate. If PE elicited the ventricular arrhythmias, the second goal of the experiment would be to compare the results of PE challenge to data collected with a 24 hour ambulatory ECG (holter monitor) in order to determine if PE administration elicited arrhythmias in dogs which had not previously manifested arrhythmias.

The PE studies were conducted on 54 puppies at 16 weeks of age and again at 24 weeks. My project involved analyzing the data that was collected from the puppies at 16 weeks of age as well as assisting with the experiments when they were repeated 8 weeks later. The experiments required anesthetizing the dogs with pentobarbital and fentanyl and then maintaining the anesthesia with a fentanyl infusion. A two lead ECG, blood pressure and respirations were recorded for ten minutes during baseline anesthesia as well as during the ten minutes following PE administration. Phenylephrine was first given at a dose of 10 g/kg and if no response was obtained, a higher dose was given (15 g/kg) after blood pressure had returned to baseline. The Holter data was collected in monthly intervals between 12 and 24 weeks of age. The dogs were classified as untreated ("unaffected") if they had ten or fewer premature complexes in 24 hours and affected if they had more than ten complexes in 24 hours.

The results of the experiments at 16 weeks of age revealed that ventricular arrhythmias could be elicited by fentanyl anesthesia (PE). The higher dose of PE produced a significant ($p=0.015$) increase in the arrhythmias when compared to baseline during fentanyl anesthesia. Three control dogs, as well as thirteen of the treated puppies, did not develop arrhythmias in response to either dose of PE. When the PE data was compared to the Holter data it was found that sixteen of the dogs that were not identified by Holter monitoring showed arrhythmias with PE administration (see accompanying figure), suggesting that PE challenge may be a more sensitive measure of predilation arrhythmias in young dogs.



The Leadership Program provided me with opportunities to address a variety of questions I had concerning alternative careers in veterinary medicine. The trip to the NIH was one of the most valuable experiences because it clearly demonstrated that a wide variety of career options are available to veterinarians in biomedical research. But without doubt, the best part of the program for me was the research. After only a few weeks, it became clear to me that what I enjoyed most about the research was the direct contact with the whole animal and being able to readily appreciate the relevance of my work. I would like to thank both the Mellon Foundation for sponsoring me this summer and Dr. Moise and members of her lab for an exciting, enjoyable and memorable summer!

Larissa Bowman - North Carolina State University

This summer I joined Dr. Vicki Meyers-Wallen's lab at the James A. Baker Institute as a Woodruff Foundation Fellow. Dr. Meyers-Wallen's research interests are inherited disorders of the reproductive tract. As my summer project, I investigated XX sex reversal in German Shorthaired Pointers (GSHP).

There are three sequential steps in normal sexual development. Initially, a mammal's chromosomal sex is determined at fertilization. Next, chromosomal sex is translated into gonadal sex. The final step, translation of gonadal sex into a phenotypic sex, is the direct consequence of gonadal development and the subsequent endocrine secretions of the fetal testis. Without testes, sexual development proceeds along the female pathway, commonly referred to as the "default pathway." Phenotypic sex formation involves the conversion of indifferent internal and external genital structures to male or female forms. What determines male development? More specifically, why do these XX dogs develop male characteristics? Current theory regarding normal male development is that a gene on the Y chromosome, Sry (Sex-determining region on the Y) is the main switch from the default pathway. At this stage, Sry mRNA is transcribed to make a protein believed to be a transcription factor that turns genes on/off to produce testes. Once testicular tissue is present, two endocrine secretions induce the male phenotype. Mullerian Inhibiting Substance (MIS) produced by Sertoli cells causes regression of the uterus and oviducts. Testosterone, from Leydig cells, directly stimulates growth of Wolffian derivatives: epididymis, vas deferens, and seminal vesicles. Testosterone also functions indirectly (upon active-site conversion to dihydrotestosterone) to induce formation of the prostate, penis, and scrotum. This is how normal male development should proceed. Sexual development does not happen this way in the GSHP model.

XX sex reversal by definition is a condition where gonadal and chromosomal sex don't agree. In the GSHP families we study, we expect ovaries in dogs with normal 78,XX karyotypes. However, affected animals develop both ovarian and testicular tissue. Surprisingly, even with testicular tissue an affected dog will have a complete uterus AND an epididymis. XXSR dogs also demonstrate enlargement of the clitoris and development of an os around the time of puberty.

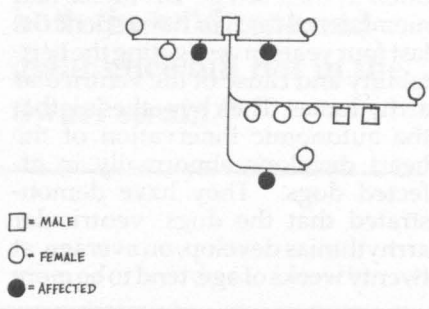
How can an animal lacking a Y chromosome develop testicular tissue? Do these animals have Sry, and if so, why? One proposed mechanism is translocation of Sry onto a non-Ychromosome. Sry cannot be visualized by karyotyping. Therefore, another, more sensitive method is required to find this gene. We chose the Polymerase Chain Reaction (PCR) because it allowed us to work with small DNA quantities.

The PCR was set up using canine-specific primers for both Sry and Hypoxanthine Phosphoribo-syltransferase (HPRT) genes. The HPRT gene, which encodes for a critical purine salvage enzyme, served as a positive control. First I identified the canine HPRT gene using human primers, cloned it, had it sequenced, and produced the required canine primers. PCR reaction conditions were chosen to maximize stringency of primer binding to target sequences. Each sample was tested in two separate reactions under identical conditions. DNA was diluted in order to use a target of ~100ng/reaction. We expected a 104bp product for Sry, and a 177bp HPRT product.

DNA from every dog demonstrated the expected HPRT product, confirming that our PCR reaction worked. The Sry product appeared only in normal males. Since the Sry gene was not detected in the genomic DNA of affected dogs, it is very unlikely that the Sry sequence is present in their genome. Therefore, testicular development in this model is probably not due to Sry gene translocation. Something else must be inducing testicular development in these GSHPs. One hypothesis is that a gene downstream from Sry in the testis differentiation pathway is spontaneously activated. This would require a mutation because testes don't develop in normal females. More research is needed to identify these genes and their functions.

I am indebted to Dr. Meyers-Wallen and Vicky Palmer for their enthusiasm, patient instruction, and for fostering a fledgling scientist. I am also grateful to Dr. McGregor for his dedication to this program which provides veterinary students with tremendous opportunities to explore career options. Special thanks to my advisors Drs. Philip Carter and Phil Sannes for encouraging my scientific pursuits. This program was the best way I could have spent my summer.

XX Sex Reversal in German Shorthaired Pointers



Natalie Burton - University of Sydney

I am currently studying for a Bachelor of Veterinary Science at the University of Sydney, and I am in my fifth and final year. Prior to my veterinary studies, I had taken courses in Psychology and Statistics, and worked as a veterinary technician in a small animal hospital. During that time I realized that veterinary science offered possibilities for an interesting and diverse career. It was for those reasons that I enrolled at Sydney University in 1989.

In 1993 I deferred my clinical veterinary studies to undertake a year of research to qualify for the Bachelor of Science (Veterinary) degree. I was engaged in epidemiological investigations of hormonal manipulation of reproduction in cattle, concentrating on risk factors associated with successful superovulation in field-based embryo transfer programs. This introduction to research inspired me to investigate career options available to veterinary graduates within research and teaching fields. Participation in the Leadership program as a Mellon Foundation Fellow provided an ideal opportunity to explore the career options.

My work this summer was conducted in the Section of Epidemiology under the guidance of Professors Yrjo Grohn and Chuck Guard, and Dr. Steve Eicker. I was involved in two projects that encompassed aspects of epidemiological study design, data collection, collation and analysis in order to answer questions about field-based problems in dairy medicine.

A successful reproductive program for a dairy herd is essential to profitability. Failure of estrus detection accounts for the greatest loss in dairy reproductive management programs for herds in which artificial insemination is used. Prostaglandin $F_{2\alpha}$ (PGF) is used commonly in dairy management to induce estrus in normally cycling cattle. The percentage of cows in a herd responding to PGF treatment, as well as the time to onset of estrus, is variable, although the precision of heat detection may be increased with the use of heat detection aids. The aim of this study was to compare the use of two mount detection aids: Kamars, a commercially available device applied to the tail base of the cow that gives a positive dye response following mounting, and tail paint, also applied to the tail base which is removed following a heat mount, within a PGF-based reproductive program. Data were collected from four local dairy herds, with a total of 527 cows enrolled in the study. To compare the efficacy of the aids we measured days to first service, which reflects the number of cows that are detected in estrus, and calving to conception interval, which reflects, in part, subsequent pregnancy rate. The data were then analyzed using survival analysis with SAS software.

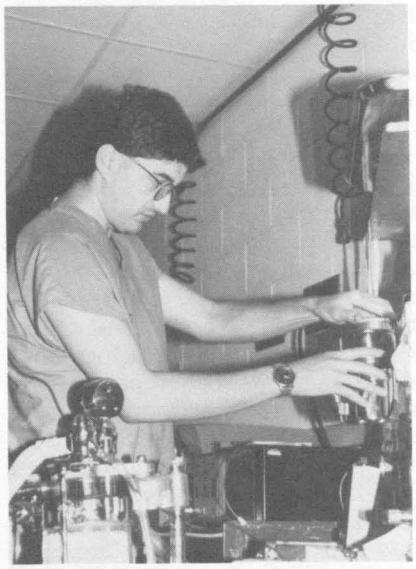
The analysis showed that treatment with Kamars resulted in more cows detected in estrus and fewer days to first service compared to tail paint. However, cows treated with Kamars had a longer calving to conception interval, indicating that Kamars may have resulted in a greater number of false positives for estrus, and subsequently more inappropriate inseminations compared to tail paint. The study enabled me to understand and develop methods in data analysis, and to work with a sophisticated data analysis software package.

The second study I participated in this summer allowed me to further develop epidemiological study skills, particularly those connected with data collection and collation. We investigated the prevalence and risk factors associated with infectious papillomatous digital dermatitis (footwarts) in dairy cattle in New York State. We conducted a randomized survey in the form of a mailed questionnaire to 750 farmers throughout New York State. Questions were asked about the incidence of the disease, seasonal occurrence, length of time that footwarts had been on the farm, size of the herd, and several management practices. The aim of the survey was to determine the prevalence of disease, and to identify risk factors that are associated with disease occurrence. We were concerned that the response to the questionnaire may have been biased by whether the disease was present on the farm. Therefore, we conducted a follow-up random phone survey to determine the prevalence independent of the survey response. Our results revealed a high prevalence of footwarts (70%) in New York State. The occurrence of disease also was associated with herd size; larger herds were more likely to be affected. Free stall housing carried a greater risk for disease compared to housing in tie stalls. The disease was more likely to be a problem in winter, and farms with disease ranked footwarts a significant problem with respect to other disease conditions occurring in the herd.

I very much enjoyed the time I spent working in the Department, and I would like to thank all those that helped to make this summer an enjoyable and challenging learning experience.

"Our results revealed a high prevalence of footwarts (70%) in New York State."

Leslie Gabor - University of Sydney



I am in my fifth and final year of the Veterinary Science program at the University of Sydney. In 1992, I spent a year in the Department of Pathology and received the degree B.Sc. (Vet). It was this initial, albeit brief, exposure to research that demonstrated to me the diverse career options that veterinary science offers its graduates - be it in biomedical research, or clinical studies. The Leadership Program at Cornell was attractive to me for I believed that it would assist in clarifying my career goals, while simultaneously affording me the opportunity to conduct research in an entirely different environment.

My project this summer was conducted in Dr. Paula Moon's laboratory in the Section of Anesthesiology. Dr. Moon's interest lies in the field of acid-base disturbances. The project focused on alternative strategies for treating metabolic acidosis. Current treatment options are limited to sodium bicarbonate therapy, fluids, and assisted ventilation with oxygen. In both veterinary and human medicine, treatment with bicarbonate is acknowledged to be inadequate, because sodium bicarbonate has numerous adverse effects. High mortalities result despite treatment.

Acidosis is rarely a spontaneous condition. It almost always is the consequence of a functional disorder - for example, end stage cardiac insufficiency, chronic pulmonary disease, or diabetes mellitus. The body compensates by increasing ventilation in the acute stage, and by renal mechanisms chronically. In the face of continuing deterioration, the acidosis itself can be life threatening.

The rationale behind bicarbonate treatment for acidosis is to buffer the excess acid. But recent reports have called attention to the deliterious effects of bicarbonate. They include decreased lactate utilization, decreased cardiac output, hypercapnia, hypernatraemia, and cerebral hemorrhage due to hyperosmolality. Because of the serious morbidity and significant mortality associated with acidosis, particularly in humans, there is an acknowledged need for alternate therapies. Compounds, such as THAM and Carbicab, are currently being tested. Our own studies were performed on acidotic dogs, treated with either THAM, bicarbonate or saline.

My project proved to be more complicated than originally anticipated. We encountered great difficulty inducing metabolic acidosis in spontaneously ventilating animals, even when the animals were obliged to breathe 7% oxygen for up to eight hours. It was only when we altered the conditions by using forced ventilation that the animals became acidotic within a reasonable period. We found that THAM and bicarbonate have equivalent buffering capacity *in vivo*, and their effects are of similar duration. Toward the end of the program, we examined haemodynamic parameters such as cardiac index, oxygen content and delivery, systemic vascular resistance, oxygen extraction as well as blood lactate and electrolyte levels before, during and after treatment.

This summer was a highlight of my rapidly ending, veterinary education. The programs organized for us, such as Career Day, the NIH visit, residency discussions and the USDA visit, to name but a few, were both enjoyable and enlightening. The generosity and hospitality of the institutions, laboratories and speakers involved were greatly appreciated. It has been a remarkably enjoyable experience living with the program participants, and it is with great sadness that I will part from the company of new friends.

I am greatly indebted to the Robert W. Woodruff Foundation which graciously funded my stay at Cornell, and to the "Queens Trust, Australia", for supporting my travel to the USA. I also wish to thank Linda Griswold and Carol Miltenberger for their efforts, and Dr. McGregor, for his commitment to this program. And I wish to thank the Anesthesiology Faculty members for their assistance over the summer, and for the interest they have shown in me and my project. Finally, I wish to extend special thanks to Paula Moon, for her patience, enthusiasm, perseverance and company.

"We found that THAM and bicarbonate have equivalent buffering capacity *in vivo*, and their effects are of similar duration."

Amanda Gaskin - University of Florida

A short time after applying to the Leadership Program, I received a phone message from Linda Griswold asking that I return her call. I was certain I had omitted some vital information in my application. Imagine my surprise when she offered me a Merck Foundation Fellowship in my first choice of interest, pathology! Needless to say, I hastily and eagerly accepted.

When I applied, I was in the process of completing my first year of veterinary school at the University of Florida (UF). I had previously received a B.S. degree in Zoology at UF and had spent a year working in a molecular neurotransmitter research lab in the College of Medicine. Pathology has become my passion. After finishing veterinary school, I hope to complete a pathology residency and perhaps a Ph.D. in cancer biology.

At Cornell I worked on the expression of retinal dystrophin in dogs with Duchenne-type muscular dystrophy (DMD) in Dr. Barry Cooper's laboratory in the Department of Pathology. The protein defective in DMD, dystrophin, is expressed at high levels in skeletal and cardiac muscle, but a series of distinct isoforms are expressed at lower levels in a variety of other tissues. These include full length (427 kD) isoforms in muscle and brain, and truncated C-terminal isoforms, Dp 116 in Schwann cells and Dp 71 in many tissues.

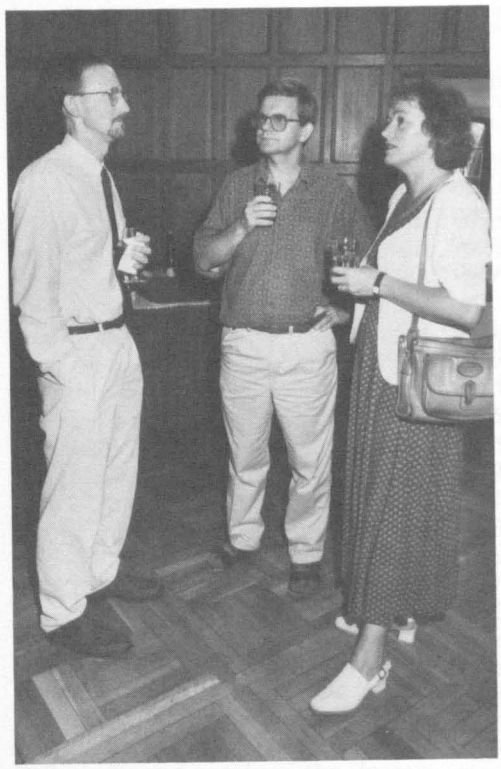
Some human patients with DMD have so-called "negative electroretinograms" (ERG), in which the b-wave is smaller in amplitude than the a-wave. Demian Dressler, a veterinary student at Cornell, and Dr. Ellis Loew performed ERGs on several dogs with x-linked muscular dystrophy (*xmd*), but failed to find any abnormalities. Other researchers have reported similar results from ERG examinations of the *mdx* mouse. Personal communication from Dr. Kathleen Fitzgerald, who has published on ERGs in DMD patients, suggests that abnormalities in the ERG occur only if the individual's mutation is in the 3′ end of the gene. In the *xmd* dog, the mutation is near the 5′ end, in intron 6. This information encouraged us to hypothesize that *xmd* dogs lack full-length retinal dystrophin, but that a truncated isoform may be present that is causally related to the observed retinal electrical dysfunction.

In this study I found an isoform of dystrophin, slightly smaller than the full-length muscle isoform in normal canine retina using Dys 1, a monoclonal antibody to the rod domain of dystrophin. Surprisingly, I have repeatedly demonstrated the same isoform in approximately equal abundance in the retina of *xmd* dogs. This result is inconsistent with our hypothesis; namely that only a truncaded dystrophin isoform would be present. We intend to study this further using additional antibodies and RT-PCR.

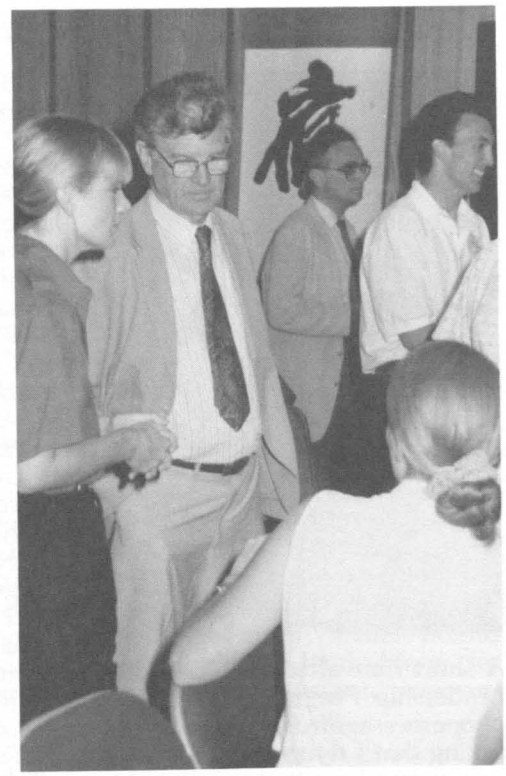
Demian and I also used a second monoclonal antibody, Dys 2, which recognizes the carboxy terminus of dystrophin. On immunostaining, Dys 2 binds to dystrophin in normal and *xmd* retina. However, Dys 2 gave only a faint signal in both normal and *xmd* retina in Western blots. It is possible that alternative splicing alters the sequence of the carboxy terminus and renders the protein unrecognizable by Dys 2. Alternatively Dys 2 may not recognize the denatured form of the protein in our blots. I plan to test this possibility in the final experiment I will conduct this summer.

I had expected a strict summer of science in the Leadership program, but my experience has been much more than that! I found most valuable the opportunities I have had to interact with students and faculty from different American and foreign universities. I am especially grateful to Yelena Kanter who was extraordinarily patient and helpful. I greatly appreciate Dr. Cooper's generosity in allowing me to work in his laboratory. Many thanks also to Dr. Douglas McGregor, Linda Griswold, and Carol Miltenberger, who organized this wonderful program and made all the participants feel so welcome!

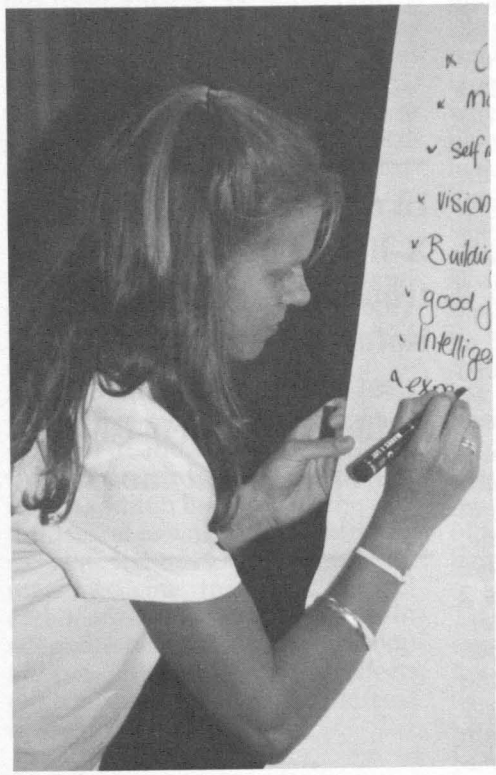
In the retinas of dogs with X-linked muscular dystrophy, "I found an isoform of dystrophin [that is] smaller than the full-length muscle isoform in normal canine retina."



Dr. Jeff Ward, Professor Yrjö Gröhn and Dr. Patricia Chisholm joined the students at the "Career Day" dinner.



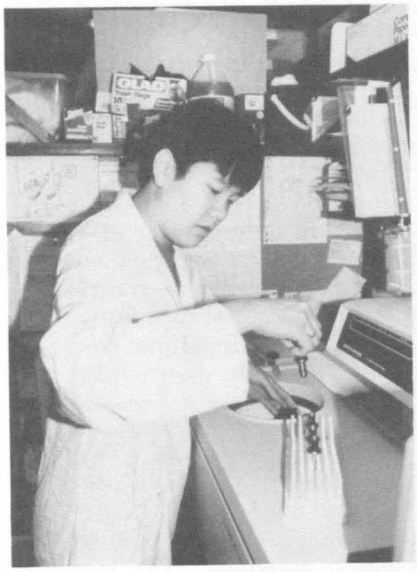
Amanda Gaskin, Dr. Peter Biggs and Mary Thompson (back) exchange thoughts about leadership.



Jantien Saltet recording characteristics of leadership.

John MacGregor at the "Welcoming BBQ."

Gwendolyn Jeun - Ontario Veterinary College



I chose to major in Zoology because of a fish tank in the biology building. I thought, what could be more fascinating than piranhas during a feeding frenzy? Four years later, I graduated with my Honours B.Sc. degree with more experience in microscopy than in fish. I stayed at the University of Western Ontario (London, Ontario, Canada) to do a Masters on the 3D spacing of nuclear components in insect epidermis. My studies continue at the Ontario Veterinary College (Guelph, Ontario Canada), where I am a proud member of the Class of 1997.

As a Woodruff Foundation Fellow, I worked with Dr. Robert Silver and his associates in the Section of Physiology. Dr. Silver's research focuses on the effects of calcium in the cell cycle in sea urchin (*Strongylocentrotus droebachiensis*) and sand dollar (*Echinaracnius parma*) oocytes. My time with Dr. Silver was spent acquiring research skills and understanding the evolution of thoughts required to do experiments we had planned for the end of the program.

Most of Dr. Silver's work is done during the spring and summer at the Marine Biological Laboratories (MBL) in Woods Hole, MA. I was fortunate to have three opportunities to visit the MBL. On the first trip, I served as an assistant in the Microinjection Short Course. It taught participants how to inject microlitre to nanolitre volumes into frog, mouse and starfish eggs. I was able to try some of the latest equipment for pulling capillary pipettes into "glass needles," used joystick-like manipulators to guide the needle to the egg and was introduced to the complex video microscopy required to view the effects of microinjecting. All this took place before I set foot in Dr. Silver's lab at Cornell!

The next two months in Ithaca were a training period. Working with echinoderms was new for me so I had to be introduced to their environment, diet and behavior. We obtained sea urchins from Maine and cultured them in laboratory tanks. I learned how to use a Hewlett Packard 8425 diode array spectrophotometer for Biuret and BCA protein assays. It helped to improve my pipetting and other basic lab skills. Mastering this technique served to illustrate the importance of achieving accurate, precise and reproducible results. Next, I learned to pour sucrose step gradients and to run a Beckman TL-100 ultracentrifuge for the isolation of membrane fractions. Practice runs used liquid nitrogen frozen and freshly spawned sea urchin eggs. I performed spectrophotometric assays using Antipyrylazo III (ApIII), a metallochromic indicator dye that binds to calcium. This method relies on the strength of the HP 8425 spectrophotometer and a kinetics program that permitted real-time assaying. With ApIII in a buffer solution, I injected 1 micromolar volume of $CaCl_2$ into the cuvette every 30 seconds over 300 seconds. ApIII binds calcium which results in a change of conformation. The change can be measured at a wavelength of 710 nm. The result is a step-wise increase in calcium absorbance over time. This particular experiment mimicked the ultimate "wet" experiments to be performed.

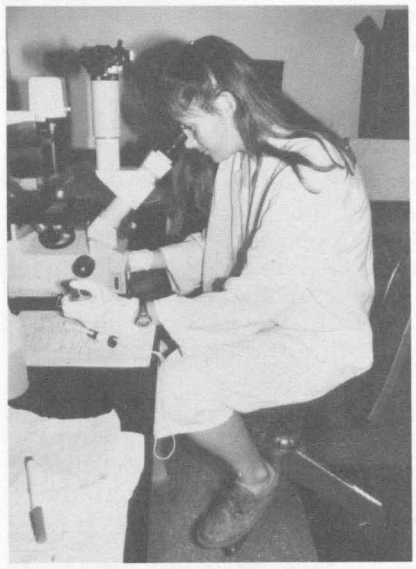
The second trip to MBL was the true test. Although my time up until then was spent learning methods with very little data collection, I discovered the importance of having had that training period. It freed me of worry about technical details and gave me more time to concentrate on the nuances of the experimental design. With Dr. Silver and his technician, we injected 1M KCl to induce the spawning of *E. parma* females and used the fresh eggs to isolate membrane fractions. The ApIII kinetic analysis was used for *in vitro* calcium assays on each of the fractions. Specifically, the membrane fractions were tested for calcium release and uptake activity. We found activity in the fraction containing endomembranes with calcium pumps and channels. These endomembranes are calcium sequestering vesicles associated with the mitotic apparatus. They are believed to be important in signaling mitosis.

On the final trip to MBL, we performed the ApIII assay but used sand dollar embryos in the two cell stage. Our preliminary results indicated that there was calcium uptake activity by the endomembranes but more work needs to be done in this area.

From these experiences, I have learned that, although it is important to pose a question in science, it is only possible to answer that question if one is equipped with the proper skills. The summer has also taught me the value of meeting people such as my colleagues in the program, the members of the scientific and veterinary communities and the support staff. I have learned much about myself in my interactions with them. Many thanks to Dr. McGregor, Linda Griswold, Carol Miltenberger, Dr. Silver, Jeb Oblak, Tim Dutta, Nadeem Shafi and my colleagues in the program for creating many wonderful memories over the last 3 months.

"Endomembranes are calcium sequestering vesicles associated with the mitotic apparatus."

Paige Langdon - VA-MD Regional College of Veterinary Medicine



I graduated from Clemson University in 1992 with a B.S. Degree in Biological Sciences, worked for a year, and enrolled in the fall of 1993 at Virginia-Maryland Regional College of Veterinary Medicine.

My introduction to research occurred at Clemson where I pursued a year long Senior Honors Thesis project investigating the neurochemical basis of aggression in crayfish. The project combined animal behavior research and high performance liguid chromatography technology to measure neurotransmitter levels in the cerebral ganglia of crayfish. I applied to the 1994 Leadership Program at Cornell University to further develop my research skills.

As a Merck Foundation Fellow, I worked in Dr. Roy Levine's lab in the Department of Pathology. Dr. Levine is studying lung development and cancer. Lung cancer is the leading cause of deaths from all cancers in the United States. Activation of specific oncogenes, such as *ras* and *erbB-2/neu*, and inactivation of tumor suppressor genes, specifically *p53*, have been associated with a substantial number of lung cancers. Mutations of the Kirsten-*ras* oncogene have been found in up to 30% of all adenocarcinomas of the lung. Malignant cellular transformation frequently involves the down regulation of specific adhesion cell surface receptors. The fibronectin receptor, α5B1 integrin, has been shown to be decreased in Kirsten-*ras* transformed rat fibroblasts.

My research involved the study of an immortalized fetal rat lung epithelial type II cell line. It has been shown that lung adenocarcinomas are derived from type II cells. Three derivatives of a progenitor cell line, 20-3, were examined. The 20-3H derivative served as a control. The 20-3T cell line originated as a colony that is morphologically distinct from the other 20-3 cells. The R4 cell line derivative was transfected with the Harvey-*ras* oncogene. All three derivatives were hygromycin resistant. My goal was to determine the cellular growth properties that were altered by introduction of the Harvey-*ras* oncogene, and to determine whether α5B1 expression was altered in these cells. Growth properties were analyzed by growth curves. Integrin expression was determined by Northern Blot analysis, Western Blot analysis and a preliminary fibronectin cell adhesion assay.

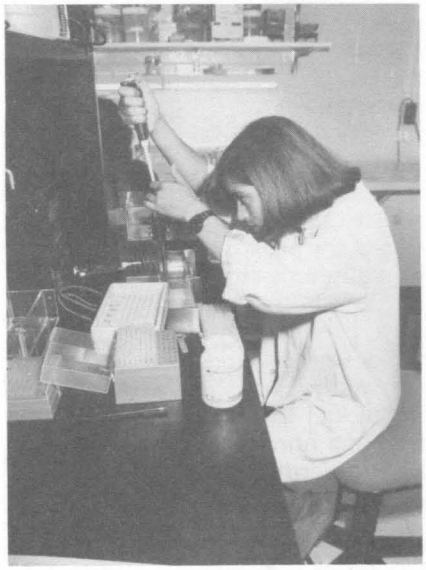
The results from growth curves indicate that while all three cell lines have similar growth rates, the 20-3T and R4 cell lines continue to grow long after the cells reach confluence. Visual observations demonstrated that the 20-3T and R4 cell lines grow in a morphologically distinct pattern from the 20-3H cell line. They do not spread out on the plate and do not respond to normal contact inhibition. Northern Blot analysis demonstrated that α5 mRNA was present in higher amounts in the R4 cell line than in the 20-3H or 20-3T cell lines. The B1 RNA was present in similar amounts in all three cell lines. Western Blot analysis determined that all three cell lines had similar amounts of the two subunits.

Preliminary research was initiated to determine if these integrin subunits were functional. A fibronectin cell adhesion assay determined that the R4 cells will attach to plates coated with fibronectin at a much higher rate than on plates lacking fibronectin, indicating that the α5B1 fibronectin receptor is most likely functional. We concluded that while transfection of the Harvey-*ras* oncogene into immortalized fetal rat lung epithelial cells results in diminished cell surface adhesion and loss of contact inhibition, it does not change the levels of the α5B1 fibronectin receptor. Further research will determine if functional differences exist between Harvey-*ras* and Kirsten-*ras* by transfecting immortalized fetal rat lung epithelial cells with Kirsten-*ras* and analyzing growth properties and integrin expression.

I would like to express my gratitude to Dr. Levine for his unending patience and sincere interest in my ideas concerning the project as well as his research assistant Tatja Hopman who led me through many of the cell biology techniques. I would also like to thank Linda Griswold and Carol Miltenberger for all the effort they put into the program- we greatly appreciated it. Dr. McGregor's support and dedication to the program made this a very worthwhile summer. The Leadership Program was very rewarding academically and socially. The visit to Washington and the many informative seminars gave me a real taste of the vast array of opportunities that exist in research. The opportunity to interact with veterinary students from around the country and world was invaluable and something I will always be grateful for.

"We concluded that while transfection of the Harvey-*ras* oncogene into immortalized fetal rat lung epithelial cells results in diminished cell surface adhesion and loss of contact inhibition, it does not change the levels of the α5B1 fibronectin receptor."

Maria Lara-Tejero - University of Madrid



As a child I looked after the most varied and unimaginable animals in La Zarza, a small village in the center of Spain. These experiences motivated me to become a veterinarian and work for the benefit of animals. Accordingly, I enrolled in a degree program at Madrid University. During my first four years, my interest was growing not only in clinical aspects of my profession (I have worked for the last two years in the large animal clinic) but also in research. Therefore, when I saw a brochure in which La Fundación Purina offered a place for a Spanish student to join the Leadership Program at Cornell University, I thought it was designed just for me. When I learned that I had been chosen, I realized that something important had happened to me.

This fall I will begin my fifth and final year at Madrid University. During this period, I will be working in the Microbiology Department on a project studying tuberculosis in cattle. When I graduate in 1995, I plan to continue my education by enrolling in a doctorate program in which I can utilize the techniques and skills that I learned this summer.

I had the opportunity this summer to participate in a research project at the Center for Canine Genetics and Reproduction headed by Professor Gus Aguirre at the James A. Baker Institute. I worked under the direct supervision of Dr. Kunal Ray. A central interest of the laboratory is to conduct research at the cellular and molecular levels with a view to elucidating the genetic and biological basis of progressive retinal atrophy (PRA). PRA is a heterogeneous group of canine retinal dystrophies. Distinct forms of PRA are recognized in different breeds of dogs. Rod-cone dysplasia type 1 (*rcd1*) is one of several canine retinal dystrophies. My project involved the development of a new, improved method for diagnosis of dogs affected with *rcd1*.

The *rcd1* phenotype is an early onset inherited retinal dystrophy segregating in members of the Irish Setter breed. It is an autosomal recessive disorder associated with degeneration and death of visual cells leading to blindness. The disease is caused by a nonsense mutation at codon 807 (**T**GG to **TA**G) in exon 21 of the cGMP PDE beta-subunit (PDEB) gene. The mutation causes premature termination of the protein by deleting 49 amino acid residues. This laboratory has developed diagnostic tests to unequivocally determine *rcd1* status. The tests employ two different strategies. First, genomic DNA from blood samples is amplified by the polymerase chain reaction (PCR). Mutation in exon 21 of PDEB gene is then determined by double stranded conformation polymorphism (DSCP). DSCP detects a point mutation but does not reveal the specific base change in the gene. In the second test, genomic DNA from blood samples is amplified by PCR using mismatch primers to artificially introduce restriction enzyme sites. The PCR product is then digested with restriction enzymes to detect the presence of the *rcd1* allele.

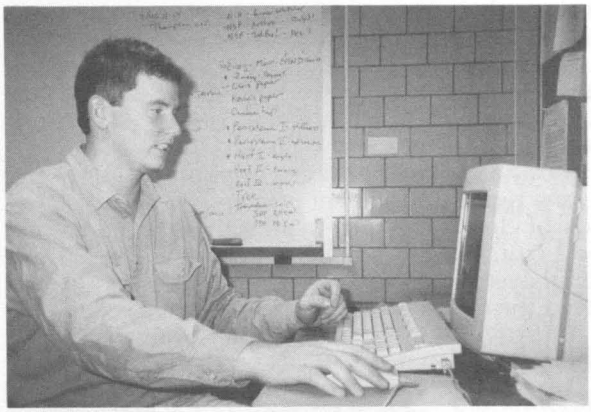
My task was to develop a reliable but more economical and less time consuming test for the diagnosis of *rcd1*. Pursuant to this goal, I used the method of allele-specific polymerase chain reaction (ASPCR). Primers were designed which include at their 3'-end the nucleotide that is involved in the mutation (e.g. **G** for normal and **A** for *rcd1* allele). Under appropriate conditions, an allele-specific primer in combination with a common primer should amplify the DNA template containing the allele for which the primer has been designed. In my experiments I used three different pairs of allele-specific primers in combination with a common primer for both alleles. PCR was done using these primers. The products were analyzed by polyacrylamide gel electrophoresis. With the first set of primers I obtained a PCR product in all samples but specificity for the wild type (normal) and mutant (*rcd1*) allele was not observed. With both the second and third set of primers, however, I obtained a PCR product which maintained the specificity for the alleles such that one could distinguish between normal, *rcd1*-affected and asymptomatic *rcd1*-carrier samples.

In short, I have successfully performed preliminary experiments to develop a new, improved diagnostic test for detection of hereditary canine blindness caused by *rcd1*. The advantage of the new test is that it involves PCR of the test samples and analysis of the PCR products by polyacrylamide gel electrophoresis only.

The Leadership Program provided an excellent opportunity to discover fields of science unknown to me in the past, thereby helping me to set my career goals. For this memorable experience, I would like to thank Dr. Aguirre, Dr. Ray, and V. Baldwin for their patience and enthusiasm. I would also like to thank members of the Leadership Program for a wonderful experience and La Fundación Purina for their confidence and financial assistance. As the first Spanish student to participate in the program, I would like to encourage the Foundation to continue its sponsorship.

"I successfully performed preliminary experiments to develop a new, improved diagnostic test for detection of hereditary canine blindness caused by *rcd1*."

John MacGregor - Cornell University



After receiving my Bachelor of Arts degree in psychology with honors from Dartmouth College in 1988, I chose to postpone my long-term career goal of becoming a veterinarian and worked as a high school teacher for five years. During that time, I married my wife Kate and completed the required course work for entrance into a veterinary school. Having finished my first year at The College of Veterinary Medicine at Cornell University, I applied for admission to (and was accepted into) the Leadership Program as a Merck Foundation Fellow. Midway through the program, Kate and I had our first child, Abigail, the first Leadership Program child to my knowledge. I would like to thank Dr. McGregor, Dr. John Bertram, my research mentor, and the rest of the Leadership students for being so supportive during this exciting time.

The goal of my project was to design a method for evaluating the impact characteristics of several different horse shoe styles. The impact, transmitted to the leg when the hoof strikes the ground, can cause horses to break down, particularly at faster gaits. Despite the widespread use of a variety of special shoes, there is little evidence that these shoes actually dampen impact. Dr. Bertram and previous leadership students had designed a system to measure deformation of horses' hooves as they ran on a treadmill by attaching rosette strain gauges to several sites on one of a horses' hooves. The minute gauges were specially prepared by hand under a microscope in order to make them sufficiently robust to withstand being attached to a running horse. Strain data was collected by a Macintosh computer using Labview virtual instruments (VIs) via a custom built cable.

The foregoing data acquisition system was modified for our current study by increasing the standard sampling rate of 883 data points per channel per second to over 2000 samples per second per channel. The modification enabled us to better access high speed loading effects at impact. A new cable system was designed and manufactured to allow the input to be switched between the six connections of each gauge.

In order to maintain consistency between trials, a single horse (Peanut) was shod with three different sets of shoes and run on the treadmill on at least two occasions for each set of shoes. The shoes used were steel, rigid polyethylene/steel composite, and aluminum/soft polyethylene composite with an alternative design. Peanut walked (1.8 m/s), trotted (3.5 m/s), cantered (6.0 m/s) and galloped (8.0 m/s, 12.0 m/s, and sometimes 14.0 m/s) on each occasion that data was collected. Each bout on the treadmill was videotaped so that the lead foot could be determined at higher gates. The data was filtered using a VI, zeroed (with the foot off the ground defined as zero strain) using Kaleidograph and analyzed using four VIs - a VI for conversion of data in bytes to voltage and then raw strain, a VI that calculated the two principal strains (tension and compression), a VI that calculated the strain angles and a VI that verified these calculations.

Most of the summer was devoted to preparing to run horses on the treadmill. Preliminary analysis of the data indicated that the basic strain wave form produced during each trial was similar. However, each shoe had a strikingly different strain pattern. For example, at the anterior lateral gauge, using steel shoes as the baseline condition, the polyethylene/steel composite shoe appeared modulate fast loading events at impact while letting the hoof deform to a greater degree. The effect was to substantially alter the direction of the principal strains. The soft polyethylene and aluminum shoe reduced both tension and compression, probably by shifting the load via design features to other parts of the hoof. Further analysis should reveal the three dimensional function of a horse's foot. It might also help determine the cause of some common foot pathologies.

I enjoyed my summer experience immensely. It was a rewarding experience to be involved in a research setting where I had some input into the design of the research process and where I had the opportunity to manufacture some of the equipment used in the project. I benefited immensely from the program. I thought the ethics discussion went well, and it was enlightening to hear viewpoints other than my own. In addition, the leadership, research and residency discussions were very informative. The program reinforced my desire to become involved in a clinical academic veterinary career. It provided me with an excellent start to what I hope will be an outstanding research background prior to entering my chosen career. Even if my plans change, this was a valuable experience, as all veterinarians can benefit from approaching clinical problems using good science. Last and certainly not least, the fellowship of the group of leadership students was quite enjoyable and the contacts that I made will serve me well as I continue my veterinary training.

"The goal of my project was to design a method for evaluating the impact characteristics of several different horse shoe styles."

Christopher Mariani - Ontario Veterinary College

This fall I will enter my third year at the Ontario Veterinary College, part of the University of Guelph, in Ontario, Canada. I completed the requirements for a B.Sc. degree during my preveterinary year, 1991-92. For the previous three summers, I had conducted research in the Department of Zoology, University of Guelph, in the area of songbird breeding biology. This led me to consider a career in research, and was integral in my decision to seek admission into the Leadership Program.

As a Mellon Foundation Fellow, I had the privilege of working with Dr. Alan Nixon in the Comparative Orthopedics Laboratory in the College of Veterinary Medicine at Cornell. Research conducted in this lab is directed toward the development of effective methods for treating articular cartilage defects in the horse. When untreated, such defects may result in osteoarthritis, long bone fractures and other complications. The Nixon group is evaluating a repair method that involves inserting a disc of fibrin laden with donor chondrocytes into the damaged cartilage. Donor chrondrocytes are obtained by harvesting articular cartilage from necropsy specimens and digesting the tissue with collagenase. Free chondrocytes are stored in liquid N_2 from where they can be retrieved, thawed and grown in cell cultures as needed.

Earlier studies showed that treatment with chondrocyte-impregnated discs results in superior healing when compared with untreated controls. More recent investigations in this laboratory have shown that regulatory proteins, such as insulin-like growth factor 1 (IGF-1) and transforming growth factor B (TGF-B) can stimulate chondrocyte growth within a three dimensional fibrin culture. The Comparative Orthopedics Laboratory is now looking for a matrix vehicle for implantation that will provide an extended supply of growth factor to implanted chondrocytes in recipient animals. Interest has focused on a number of surgical polymers that might be used to secure the fibrin disc in place, while gradually releasing growth factor to the cells.

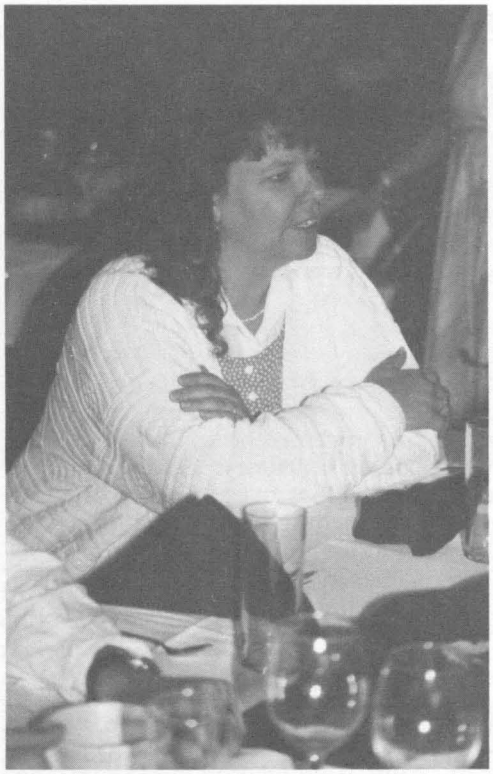
One of my projects this summer was to try and incorporate IGF-1 into various polymers. The amount of growth factor eluting from these materials was quantified over time by HPLC analysis. I tested three polymers, Polyglactin 910 (Vicryl), Polydioxanone (Orthosorb Pins), and Poly-L-lactide (Biofix Tacks) in this manner, as well as placing IGF-1 into the fibrin disc itself. The polymers were treated with 10 µg/ml of IGF-1 for 48 hours, but none showed detectable uptake or subsequent release of IGF-1 over the next 4-5 days. Fibrin discs, formed by combining thrombin and fibrinogen in the presence of 10 µg of IGF-1 per disc, gave more promising results. IGF-1 was released in gradually declining amounts from the discs over a period of 14 days. Studies to test additional polymers that might augment growth factor release from fibrin are ongoing.

A second project in which I became involved dealt with the genetic labelling of implanted chondrocytes so that the relative contribution of donor and recipient cells to the healing process could be evaluated. Chondrocytes were "labelled" with an avian retrovirus (Duck Spleen Necrosis Virus - SNV). A replication defective strain of SNV has been engineered to carry the bacterial gene lacZ. Upon infection of a chondrocyte, the "labeled gene" is integrated into the cell's genome. The labelled gene is inherited by daughter cells thereby providing the basis for identifying the cell lineage. LacZ encodes for B-galactosidase, which, after the addition of the substrate, X-gal, turns cells possessing the enzyme blue.

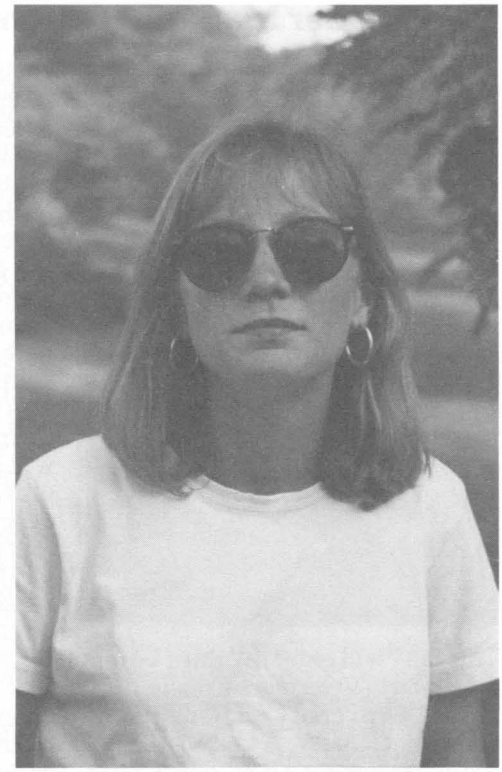
I achieved some success in the labelling of chondrocytes using a variety of techniques, and established which procedures resulted in a higher labelling efficiency. Additional techniques that may improve our success include electroporation of the chondrocytes and the use of liposomes to encapsulate the retrovirus. Experiments using these approaches are currently being explored.

I found the Leadership Program to be an extremely enjoyable experience, both socially and academically. It allows students to gain laboratory and often clinical experience, and also to forge life-long friendships with other students from around the world who have similar interests and aspirations. The field trips to the NIH and USDA were very informative, and along with numerous discussion sessions such as Career Day, helped me decide on future career development paths. I would highly recommend the program to anyone contemplating a research career. I would like to thank the Mellon Foundation for making it possible for me to attend this program, Linda Griswold and Carol Miltenberger for a fantastic job in coordinating the program, and Dr. McGregor for having the vision to conceive the idea, and for having such confidence in all of us.

"I achieved some success in the labelling of chondrocytes using a variety of techniques."

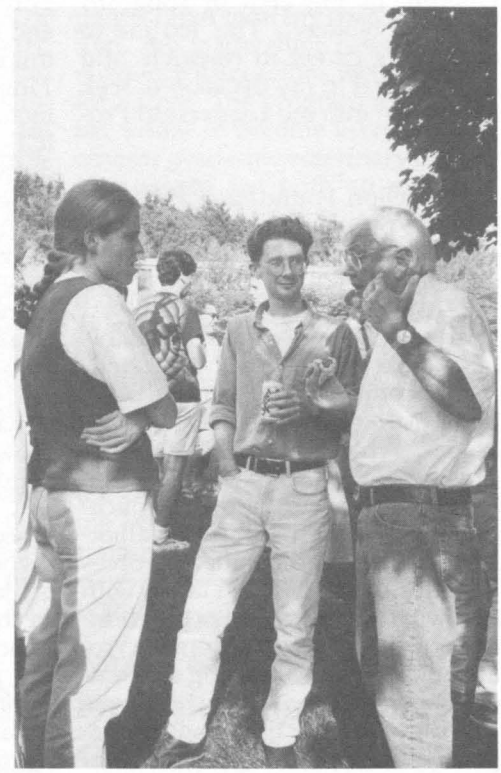


Linda Griswold made many of the arrangements for the program.



Carol Miltenberger served as Program Coordinator again this year. She is now pursuing a career in financial management.

Ms. Sue Baptiste (McMaster University) engaged the students in a discussion of group learning.



Mary Thompson talking with 1992 Leadership Program "graduate," Dr. Steven Davies and Professor Robert Lewis.

Sonia Mumford - North Carolina State University

I am one of those people who grew up knowing that I only wanted to be a veterinarian. From an early age, I started working towards that goal. As an undergraduate at the University of North Carolina at Chapel Hill, however, I became involved in an environmental conservation group. As I became aware of the incredible environmental problems we face, I nearly gave up my dream of becoming a veterinarian in order to become a full-time, grass roots organizer. But, I realized that I could facilitate environmental conservation as a veterinarian. Thus, I decided to become a wildlife veterinarian. As I explored this further, I discovered how little we know about non-domestic animals, especially aquatic animals. I am especially interested in the immune systems of these animals. As we better understand how their immune systems work, we may be able to augment their capacity to resist disease. I hope to adapt techniques developed for more traditional animals and apply them to marine animals.

This summer, I received a Merck Foundation Fellowship which enabled me to work with Dr. Paul Bowser in the Department of Avian and Aquatic Animal Medicine. My project involved adapting a rat immunohistochemical kit for use in fish. We used a monoclonal antibody targeted at proliferating cell nuclear antigen (PCNA) in Walleye dermal sarcoma. The Walleye dermal sarcoma is a seasonal tumor caused by a retrovirus. The tumors regress simultaneously in the spring and summer. If one could elucidate the mechanism by which the tumor regress, it might provide insight into pathogenic mechanisms associated with other retroviruses, such as the one that causes AIDS.

PCNA is a biomarker in cells that are rapidly proliferating. Cells staining positive for PCNA are predominantly in S phase of the cell cycle or are undergoing DNA excision repair. PCNA is highly conserved in that it is found in mammals, plants, amphibians, protozoa, and even viruses.

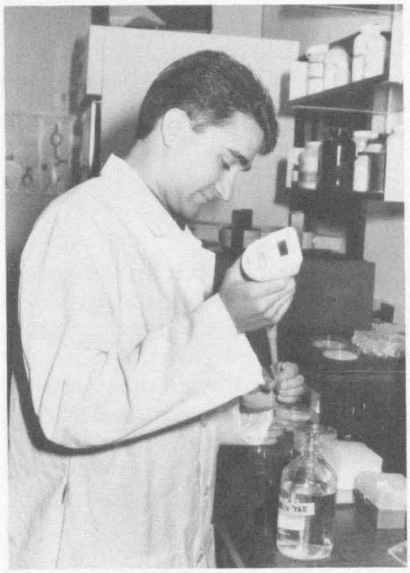
I found that with some modification, the commercial kit could be used successfully on fish. In addition to the Walleye dermal sarcoma, a rainbow trout hepatoma, and a lake trout lateral line tumor also showed a proliferative signal. The numbers of cells staining positive for PCNA accorded with their cellular proliferation rates; but more research must be done to validate the technique and compare its utility with established methods for measuring cell proliferation. There are several advantages of the PCNA over current methods that utilize tritiated thymidine (^{3}H-TdR) or bromodeoxyuridine (BrdU). One of the most important of these is that PCNA can be used in archived tissue. Thus, retrospective studies can be performed in addition to prospective studies. PCNA has another important advantage over ^{3}H-TdR in that it does not require the use of a radioactive reagent which can be harmful to the researcher and the environment. Also, PCNA does not require the surgical implantation of osmotic pumps for infusion.

PCNA has potential value as a diagnostic and/or prognostic tool. Its presence can be detected in several known fish tumors. The next step is to stain smaller tumors and preneoplastic lesions to detect the earliest cell proliferation signal. In the Walleye, it would also be interesting to corroborate PCNA with in situ hybridization for viral nucleic acids. Medical applications for PCNA are already being investigated. Proliferation rates as detected by PCNA have been shown to be a valuable prognostic tool in certain tumors such as malignant melanoma in humans.

I enjoyed my research this summer. I very much appreciate the privilege of working with Dr. Paul Bowser, Greg Wooster, Dean L'Amoreaux and others in the Department of Avian and Aquatic Animal Medicine. Thanks to Dr. Dana Stoffregen of the Pharmacology Department, who patiently answered the twenty questions a day I asked him. Also, thanks to Dr. Philip Carter of NCSU for his continued support. Lastly, I would like to express my appreciation to Dr. Douglas McGregor in making this summer possible. I gained much from this program, because I was doing exactly what I wanted to do. I wanted to get my feet wet in working with aquatic animals and continue to develop my research skills. In addition, I will write my first scientific paper from my summer's research and submit it for publication.

"The numbers of cells staining positive for PCNA accorded with their cellular proliferation rates ..."

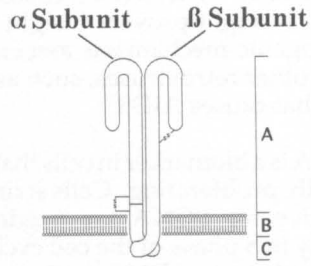
Jeffrey Nunez - Louisiana State University



My introduction to academic research began as an undergraduate with Dr. Gary Winston in the Department of Biochemistry at Louisiana State University. I was involved in a two year study of benzo[a]-pyrene metabolism in the American alligator, *Alligator mississippiensis*. This experience, coupled with the advice of several faculty members, motivated me to seek the Doctor of Veterinary Medicine and Ph.D. degrees with the ultimate goal of pursuing a career in academic or industrial research. Following graduation from LSU in 1992 with B.S. degrees in Microbiology and Biochemistry, I enrolled in the School of Veterinary Medicine at the same institution. I am now beginning my third year of study. During veterinary school, I managed to broaden my research experience by participating in summer research programs at Auburn University School of Veterinary Medicine and, this summer, at Cornell. As a Woodruff Foundation Fellow, I conducted research on integrins under the direction of Dr. Jun-Lin Guan and his postdoctoral student, Dr. Paul Appeddu in the Cancer Biology Laboratories of the Department of Pathology.

Integrins are a class of cell surface heterodimeric receptors composed of an α and β subunit, each having a large extracellular domain, a membrane spanning region and a short cytoplasmic domain. (See Diagram).

Integrins mediate cellular interactions with the outside environment by binding extracellular proteins, integral membrane proteins or other cells.



A: Large Extracellular Domain
B: Membrane Spanning Region
C: Short Cytoplasmic Domain

In addition to their functions in cell-to-cell or cell-to-matrix attachment, integrins can act as signal transducers by conveying information into or out of the cell. Of particular interest to my project is the cytoplasmic portion of the integrin β-subunit (β_{cp}). β_{cp} has a critical role in triggering intracellular signals initiated by the binding of ligands to integrins. It is hypothesized that intracellular proteins bind β_{cp} and mediate the downstream signaling events. One of the goals of this lab is to identify these proteins by the use of a yeast two hybrid system. In the two hybrid system, the protein of interest (β_{cp} in this case) is ligated into the plasmid pGTB9 adjacent to the binding domain of Gal4. Expression of the hybrid plasmid in yeast cells yields a fusion protein consisting of β_{cp} and the binding domain of Gal4. Yeast cells bearing pGBT9 are subsequently transformed with plasmids pGAD424 containing a cDNA library cloned adjacent to the activating domain of GAL4. Expression of pGAD424 also produces a protein consisting of the activating domain of GAL4 fused to a protein component of the DNA library (called Protein X). If Protein X encoded by one of the library plasmids interacts with β_{cp}, the binding and activating domains of GAL4 are brought into close proximity allowing transcription of the lacZ gene and production of β-galactosidase. β_{cp} interacting proteins can be identified by assaying yeast cells for production of β-galactosidase, which cleaves X-gal substrate yielding a blue color.

My project this summer involved fusing of cDNA's encoding wild type β-integrin as well as seven mutant β-integrins to the DNA binding domain of Gal4 in the pGBT9 plasmid. These seven mutant β-integrins contain point mutations of selected amino acid residues that may be useful in elucidating how proteins interact with β_{cp}. After optimizing the polymerase conditions, amplification of the β_{cp} fragments was achieved by the Polymerase Chain Reaction (PCR). PCR mutagenisis of base pair 2357 was also required to introduce a MsI restriction site which allowed for ligation of the β_{cp} cDNA into pGBT9. Once the hybrid pGBT9 plasmids were constructed, they were amplified in *E. coli* DH5α cells. This involved transforming *E. coli* cells, plating them on selective media, and performing miniprep DNA isolations of bacteria that grew on the media. Diagnostic digestion of the minipreps with PstI and HindIII was then performed and separated on agarose gels to identify colonies harboring the pGBT9:β_{cp} construct.

I've found the Leadership Program for Veterinary Students to be outstanding. I especially enjoyed the research experience, the tours to the NIH and USDA, as well as the frequent seminars on career opportunities in veterinary medicine. In addition, the chance to meet and interact with veterinary students from different parts of the country as well as from around the world provided me the opportunity to forge lifelong friendships.

"After optimizing the polymerase conditions, amplification of the β_{cp} fragments was achieved by the Polymerase Chain Reaction (PCR)."

Jeffrey Phillips - Tufts University

When I embarked on my veterinary education at Tufts School of Veterinary Medicine, my goal was to not only study at the veterinary school, but also to engage in research at the Sackler Graduate School of Biomedical Sciences. My objective was to obtain both a clinical and a research oriented education. After completing my second year of veterinary school, I will commence work on a graduate degree in Genetics at the Sackler School.

My purpose in applying to and participating in the Cornell Veterinary Leadership Program was to gain additional experience in veterinary research in an unique and interactive educational setting. Through the generosity of the Merck Foundation I was able to conduct research on parvoviruses under the direction of Dr. Colin Parrish, whose research spans a wide variety of topics in the field of virology.

My particular project involved an analysis of the capsid protein structure of canine parvovirus (CPV) and its assembly/disassembly processes. CPV, like all parvoviruses, consists of a single strand of DNA contained within a protein capsid comprised of multiple copies of a combination of the viral proteins VP-1, VP-2. Parvoviruses are highly stable, isometric, nonenveloped "particles." In order to quantify their stability, the disassembly of the viral protein capsids was studied under several different conditions. Purified viral protein capsids were subjected to heat in the presence of SDS followed by electrophoresis in SDS-polyacrylamide gel electrophoresis. From the results of these experiments, the temperature dependence of viral protein denaturation was determined. Other procedures used to examine the disassembly process included the use of urea and the manipulation of pH.

I also conducted studies aimed at determining the cellular location of viral protein capsid assembly. It is currently believed that the viral proteins (VP-1, VP-2) must be transported into the nucleus of host cells prior to their final assembly into protein capsids. In order to test this notion, cells were separated into cytoplasmic and nuclear fractions and then run on sucrose gradients to seperate unassembled capsids (monomers, dimers, etc.) from those that were fully assembled. The gradients were fractionated and then analyzed via Western blots. The results clearly suggested that the viral protein capsid was primarily assembled in the nucleus. As an adjunct, transfected cells, which express the wild type protein capsid, were probed using immunofluorescence using different antibodies and conditions, to compare the amounts of fully assembled protein capsids in the cytoplasm or the nucleus.

Still another area I investigated involved the cloning of viruses in which various mutations were created. I was able to successfully create a plasmid clone containing two additional restriction enzyme sites, and placed that clone in an expression vector (pCDNA1-neo) so that mutant viral proteins could be recovered and their properties studied.

In summary, I was very pleased with my summer experience. I was able to receive formal research training in the field of virology and also learn many of the current techniques used in molecular virology. The work I have been engaged in has provided me with seeds for my future graduate training in genetics. In addition, the excursions to the NIH and USDA gave me an opportunity to see the quality of work at our government financed research institutions. I would like to thank Dr. McGregor, Linda Griswold, and Carol Miltenberger for all their hard work in making this a very successful summer research and leadership training program. Finally, I would like to thank all my research associates in Dr. Parrish's lab who provided their invaluable help, and of course, Dr. Colin Parrish who is both a brilliant scientist and excellent teacher.

"I was able to successfully create a plasmid clone containing two additional restriction enzyme sites, and placed that clone in an expression vector (pCDNA1-neo) so that mutant viral proteins could be recovered and their properties studied."

Julie Pomerantz - Cornell University

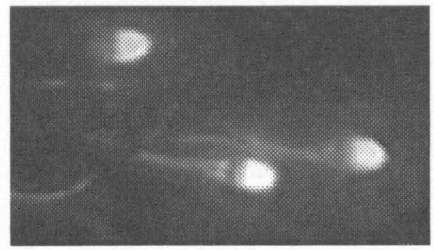
As a student at Cornell University I have had the good fortune to become involved in many areas of veterinary medicine: academic, clinical, and research. My first experience in research came before I entered veterinary school, when I worked for Dr. Peter Nathanielsz in the Laboratory for Pregnancy and Newborn Research. Under the direction of Dr. Nathanielsz and Dr. Thomas McDonald, I undertook two projects, one related to the growth of newborn lambs and the other an immunohistochemical study of corticotropin releasing factor (CRF) in the adrenal glands of the ovine fetus and neonate. Now, as I am entering my second year as a veterinary student, I am beginning to fully appreciate the interdependence of the many disciplines encompassed by veterinary medicine and particularly the importance of research.

My research this summer also was in the field of reproductive biology. As a member of Dr. Barry A. Ball's laboratory, my goal was to examine the distribution of cell membrane glycoproteins in equine spermatozoa. Membrane glycoproteins have important roles in cellular interactions, such as that between the spermatozoon and the oocyte. It has been previously demonstrated in other species that the distribution of spermatozoal cell membrane glycoproteins is not static, but changes as the cell matures in the epididymis and during capacitation and binding to the zona pellucida. One technique used to detect these molecules capitalizes on their capacity to bind lectins. Lectins are naturally occurring proteins derived from a variety of plant and animal sources. They bind with high specificity to particular oligosaccharides. Lectins conjugated to fluorescein-isothiocyanate (FITC) are frequently used as probes for detecting sugar residues by epifluorescence.

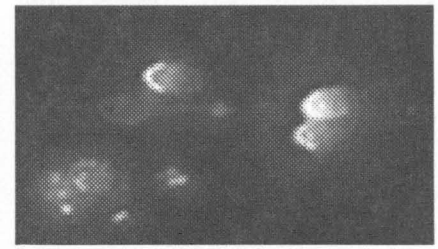
In the study I undertook, stallion semen was collected by artificial vagina and a preliminary examination was made to determine the concentration and relative motility of the spermatozoa. The semen was then diluted and washed through 45% Percoll to remove the seminal plasma. The membrane integrity of the spermatozoa, an indicator of their viability, was determined by the ability of the cells to exclude Hoechst 33258, a fluorescent dye. The spermatozoa were then incubated with solutions of eight different FITC conjugated lectins with varying oligosaccharide specificities. Spermatozoa, with labeled cell membrane glycoproteins were then examined as wet mounts by fluorescence microscopy. Various labeling patterns were observed and quantified (see accompanying figures). Specific labeling patterns were found for some lectins while for others the patterns were inconsistent. For two of the lectins, minimal binding was observed. There were also significant differences in the prevalence of the various binding patterns among Hoechst "positive" and Hoechst "negative" cells. Negative controls were prepared by preincubating the lectins with excess oligosaccharide of the appropriate specificity, a procedure that inhibited their binding to spermatozoa. Future studies will capitalize on these lectin binding patterns to examine changes in the distribution of equine sperm cell membrane glycoproteins during capacitation and the acrosome reaction.

Participation in the Leadership Program allowed me to examine a new area of interest, and it has been of great help to me in defining my professional goals. Among the many activities this summer, I especially enjoyed our visits to the NIH and the USDA. I also found the career discussions with faculty to be enlightening. I am primarily interested in pursuing a career in clinical medicine, and look forward to the opportunity to become an active member of my profession by engaging in clinically relevant research.

I am indebted to Dr. Barry A. Ball for allowing me to work in his laboratory, and to Dr. Ina Dobrinski, Dr. Philip G.A. Thomas, and Ms. Marketa Lillard for their patience and encouragement. I would also like to express my appreciation to Dr. Douglas McGregor, Ms. Linda Griswold, and Ms. Carol Miltenberger for organizing the Leadership Program. Finally, I would like to thank the Mellon Foundation for its generous support of my fellowship.



1



2

Binding of lectins to equine spermatozoa. 1) Specific binding of Peanut Agglutinin to the anterior sperm head. 2) Specific binding of Ricinus Communis Agglutinin I to the apical ridge.

Stacy Pritt - Washington State University

After graduating *summa cum laude* in Biology from the California State Polytechnic University, Pomona, I entered veterinary school at Washington State University. My interest in research arose during my three years as a research technician with the Huntington Medical Research Institutes in California. Through neurosurgeries, nuclear magnetic resonance, and magnetic resonance imaging, I investigated aspects of ischemia in piglets. I also was responsible for the care of the animals and construction of surgical and drug protocols. This experience also piqued my interest in laboratory animal medicine. Currently, I wish to pursue advanced training and board certification in laboratory animal medicine after I graduate in 1997.

My research interests and extensive involvement in student government while an undergraduate and veterinary student, motivated me to seek admission into the Leadership Program. The combination of research and consideration of the characteristics and responsibilities of leadership provided a stimulating summer.

My research project was pursued in Dr. Robert Gilbert's laboratory. It included aspects of theriogenology, immunology and hematology. The main thrust of the project was to determine the pathogenesis of vesiculitis in bulls. Previous research by Dr. Gilbert and his associates had demonstrated inhibitory effects of seminal plasma on neutrophil function. Unknown constituents of bovine seminal plasma inhibit the oxidative burst in neutrophils. Seminal plasma also decreases neutrophil aggregation and is cytotoxic to neutrophils. This inhibitory effect is logical because of the need for protecting sperm once they are in the female reproductive tract. Polyunsaturated fatty acids in their membranes render them vulnerable to the oxidative burst.

The unknown constituents of seminal plasma that affect neutophils are believed to originate in the prostate or vesicular glands. Our research focused on the vesicular gland of the bull because of the frequent occurrence of chronic and persistent infections of the gland. Vesiculitis is difficult to treat and spontaneous recoveries rarely occur in the adult bull, even in the presence of normal neutrophil infiltration. This could be explained by an adverse effect of glandular secretions on neutrophil function. The vesicular glands are mainly responsible for secreting major amounts of seminal plasma and prostaglandins.

We examined the effects of vesicular gland secretions on neutrophils with a view to elucidating the pathogenesis of vesiculitis. To this end, bovine vesicular gland epithelium was grown in cultures. Since vesicular gland secretions are testosterone-dependent, we grew the cells as monolayers in the presence or absence of testosterone or dihydrotestosterone. Freshly isolated neutrophils from mature cows were exposed to conditioned media from the various cell cultures to ascertain possible cytotoxic effects or inhibitory effects on the oxidative burst and activation of neutrophils.

Three tests were used. The first utilized the reduction of ferricytochrome C to quantify superoxide anion production. The second was the Trypan Blue Exclusion test. Trypan Blue stains dead cells in which membrane integrity is lost. Shape change analysis of neutrophils exposed to zymosan activated plasma, a source of complement fragments, was the basis of the third test. It allowed for measurement of the degree to which the cells are "activated" over a period of two minutes.

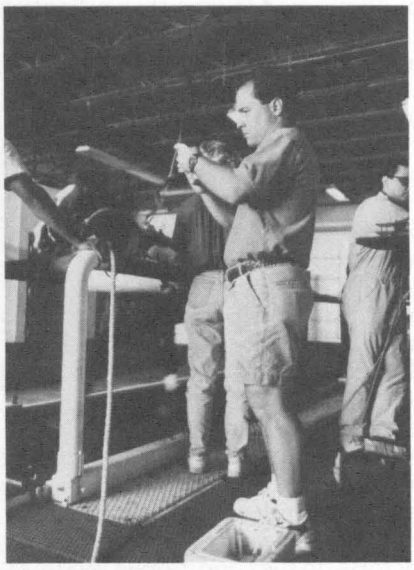
Statistically significant differences were not observed when neutrophil activation or death rates were compared in groups treated or not treated with the epithelial cell conditioned media in the cytochrome C reduction and Trypan Blue Exclusion tests. In the shape change analysis test, however, neutrophils exposed to epithelial cell media did exhibit significant increases in activation. The latter suggests that a factor(s) in the conditioned medium had a demonstrable effect on neutrophil function as expressed in the level of cell activation.

Further research will be needed to verify these findings. If the epithelium does not influence neutrophil function (or if it stimulates rather than inhibits the cell), then other sources for the persistence of vesiculitis and the inhibition exhibited by seminal plasma will need to be investigated.

This summer has given me a new appreciation for academic research and the invaluable experience of visiting a different veterinary college. I am grateful to Dr. Robert Gilbert for his time. Thank yous are also due to Dr. Alix Scheytt for her assistance and the managers of Cornell's dairy herd. And, I would like to extend a special thank you to the Merck Foundation, for providing me with the fellowship that enabled me to participate in the program.

Our findings suggest "that a factor(s) in the conditioned medium had a demonstrable effect on neutraphil function as expressed in the level of cell activation."

Bradley Russ - VA-MD Regional College of Veterinary Medicine



Prior to attending veterinary school, I received a BA degree in Economics and a MA in Finance, each concentrating in areas of biotechnology finances and management. Following graduate school I went to work for The Johns Hopkins University School of Medicine. The experience rekindled my interest in animals and medicine, and I soon found myself enrolled in science courses and embarking on a new career in veterinary medicine. I continued to work at Johns Hopkins in its Division of Pulmonary and Critical Care Medicine studying respiratory physiology in animals and humans. Specifically, I participated in the investigation of upper airway mechanics in cats and also the development of a chronic dog model of sleep apnea and its effects on cardiovascular performance over long periods of time.

At Cornell University, it was my good fortune to work with Dr. Dorothy Ainsworth in the Department of Clinical Sciences. Our research investigated functions of the equine diaphragm with the ultimate goal of determining whether diaphragmatic dysfunction (fatigue) may limit equine performance. A recent study has hypothesized that while the diaphragm is the primary inspiratory motor at rest it has a comparatively minor role during exercise, proposing that during exercise ventilation occurs secondary to locomotory-induced movement of the abdominal viscera (visceral piston) rather than diaphragmatic contractions per se. We endeavored to disprove this hypothesis by conducting exercise experiments that examined the role of the diaphragm in meeting the ventilatory demands of the horse during treadmill exercise. Ultimately this research will have direct applications to the development of treatment strategies for horses with lower airway obstruction, such as heaves or bronchiolitis. Such diseases place a resistive load on breathing and may cause the diaphragm to fatigue, inhibiting exercise performance. Thus, the long term goal of the project is to develop therapies aimed at improving the efficiency of the respiratory muscles and thereby increasing exercise performance under strenuous conditions.

Our studies this summer used four chronically instrumented horses that had been acclimated to treadmill exercise. Each horse had costal diaphragmatic bipolar electrodes surgically implanted to measure electromyographic (EMG) activity. Mechanical output of the diaphragm (transdiaphragmatic pressure) was measured by pressure transducers placed in the thoracic esophagus and the stomach. Heart rate, arterial blood gases, and stride frequency were also monitored as each horse performed incremental exercise tests.

Results thus far have shown that diaphragmatic EMG activity increases linearly with exercise intensity and that electrical activity is correlated with mechanical output (transdiaphragmatic pressure). These findings suggest that diaphragmatic activity plays a significant role in meeting the increased ventilatory demands of exercise in the horse, although the impact of the visceral piston in generating airflow could not be determined. However, in the subsequent exercise trials, the lab hopes to explore this question by measuring diaphragmatic activity while horses exercise on a 10° slope. Such an exercise protocol should "dampen" the effect of the visceral movement, giving a better assessment of true diaphragmatic activity during exercise.

When I came to Cornell, I was quite unsure what possibilities exist for a veterinarian in the field of research and academics. By being given the opportunity to speak with respected and successful individuals from a variety of institutions, I was able to gain a better appreciation and understanding of the contributions a veterinarian can make in the research community. The program encouraged each of us to examine our personal goals and aspirations and to acquire as much information about career paths as possible. I would like to thank Dr. McGregor and his staff for their constant support and attention. I would also like to thank my mentor, Dorothy Ainsworth, for her guidance and confidence and for providing an environment where I could work and learn without hindrance. Most importantly, this program established lasting friendships and fond memories that made it a summer well spent.

"... diaphragmatic EMG activity increases linearly with exercise intensity and electrical activity is correlated with mechanical output (transdiaphragmatic pressure)."

Jantien Saltet - University of Sydney

As a child, my dream career was to be a country vet, but when it came to applying to university, I wasn't so sure it was the right choice. After seven months full-time work as a vet nurse, I decided that I really was interested and accepted my place in veterinary science at Sydney University. I took a year off between finishing school and starting university, however. The year gave me more confidence about my choice, as I traveled and had a wide range of experiences.

After finishing the third year of my B.V.Sc, I again decided to take a year off - in order to pursue other interests. I applied for the Leadership Program for Veterinary Students and was awarded a Woodruff Foundation Fellowship. This offered me a chance for both change and travel, as well as experience and opportunity. As part of the program I worked in Dr. Richard Cerione's laboratory in the Department of Pharmacology at Cornell. My supervisor, Dr. Jim Flanders, designed the project I was to work on. It was along the same line as the topic he had selected for his Ph.D. research, "Signal Transduction involving Tyrosine Kinases in Human Breast Cancer Cells." My task was to evaluate the presence of certain receptor and non-receptor tyrosine kinases in human lung cancer cells.

Tyrosine kinases are proteins that catalyze the transfer of phosphate groups from ATP (most commonly) to tyrosine residues on proteins (called phosphosubstrates). By doing so, they alter the activity of the substrate. Tyrosine kinases exist in normal cells, at baseline levels. Their activation in a regulated manner is believed to be essential for normal cell function. Tyrosine kinases are over-expressed in various cancer cell lines, and it is thought that this overexpression may have a role in oncogenesis. Few studies to date have investigated overexpression of tyrosine kinases in lung cancers. My project afforded an opportunity to do this.

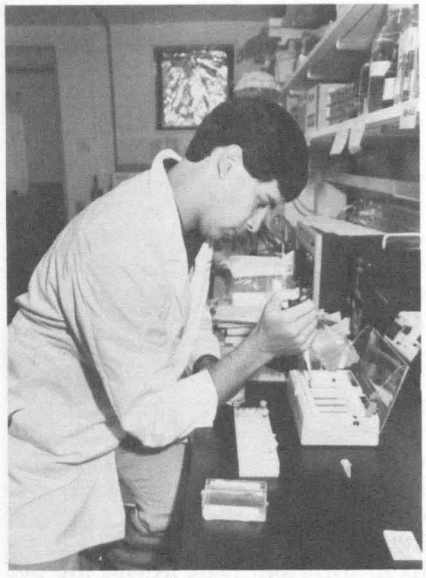
The five tyrosine kinases I examined are all products of oncogenes. They include four members of the transmembrane receptor tyrosine kinase family: the epidermal growth factor (EGF) receptor, erbB2/neu, erbB3, and erbB4, and one non-receptor tyrosine kinase, src. The subjects of my study were six lines of human lung cancer cells that I received frozen in liquid N_2. My first task was to thaw the cells and maintain them in culture under sterile conditions. The cell lines included: A427, H596, CALU-1, CALU-6, SKLU-1 and SKMES. All were derived from different human, non-small cell lung cancer cell lines (NSCLC). The latter include adenocarcinomas, squamous cell carcinomas, large cell carcinomas and mesotheliomas.

To ascertain whether the cells contained tyrosine kinases I needed sufficient numbers to yield between 750-1200 µg of protein. Similar amounts of protein also were needed from positive and negative controls. I lysed the cells with NP40 lysis buffer and isolated the proteins, free from membrane material, then used the lysate to perform immunoprecipitations, using antibodies directed against the specific tyrosine kinases mentioned. This step eliminated most of the other proteins in the cell. I then electrophoresed the immunoprecipitates of my lysates, as well as controls and "pure" lysate, on polyacrylamide gels to separate the remaining proteins by molecular weight. The next step was to transfer the proteins to a sheet of Immobilon, as the gel is easily destroyed. Finally, I used the transfer to perform a Western blot, using the same antibody and developed the image as an autoradiograph. The procedure allowed me to compare the level of protein in the lung cancer cells, to that in a cell line known to overexpress the protein, as well as one known to have only base line amounts.

Unfortunately I was unable to do much of what was planned, because the cells grew very slowly initially, due to a number of factors, including cell density on the plate, medium suitability and pH. Only during the last couple of weeks did I have sufficient cells to run my experiments. Despite this setback I learned a variety of new techniques and concepts at a more detailed level, than could ever be expected during my formal education. More importantly though I gained insight into the world of research, which I thoroughly enjoyed. I became acquainted with a wide range of career possibilities and opportunities, some of which I had previously not thought about. I gained much from the experience, both in and out of the lab, and I will never forget it.

"Few studies to date have investigated overexpression of tyrosine kinases in lung cancers. My project afforded an opportunity to do this."

Ralph Sellman - University of Tennessee



My desire to pursue a career in veterinary medicine has been a lifelong ambition stemming from a love of animals, and the veritable zoo — ranging from fish to Siamese cats to parrots — which I somehow persuaded my parents to collect. My undergraduate years while obtaining a B.S. in Biology from the University of Redlands in sunny California encouraged my interest in research. This interest was strengthened by my senior research thesis, which involved behavior and breeding studies to determine if we could expand the range of an endangered form of *Gasterosteus aculeatus* (the three-spined stickleback) by introducing them to a proposed artificial wetlands waste-water management facility.

After gaining entrance to the University of Tennessee College of Veterinary Medicine, I decided to seek further research experience there last summer, where I performed radioligand binding studies to determine the concentration of PGE2 receptors in the canine iris-ciliary body. The experience focused my desire to explore research in canine ophthalmology (the area I have become interested in) as a possible career. I applied to the Leadership Program in order to obtain more experience and counseling so that I might better make an informed decision as to which career path in veterinary medicine I will pursue.

The Woodruff Foundation Fellowship I received this summer enabled me to work with Dr. Bennett Hershfield at the James A. Baker Institute for Animal Health. Dr. Hershfield is engaged in gene mapping in dogs. Contemporary molecular methods are applied to investigate genetic similarities between dogs and humans, the genetic basis of differences between dog breeds, and questions regarding inbreeding and outbreeding in dog pedigrees. Dr. Hershfield is currently working with dog pedigrees for hereditary retinal degenerations.

My project goal was to map the XLPRA (X-linked progressive retinal atrophy) gene. XLPRA is a novel hereditary retinal degeneration found to date only in Siberian Huskies. All other forms of PRA are inherited in an autosomal manner. Thus, XLPRA is the only existing animal model for X-linked retinitis pigmentosa, a similar retinal disorder that occurs in humans. The specific mutation responsible for this disorder in dogs is currently unknown and is the subject of ongoing research.

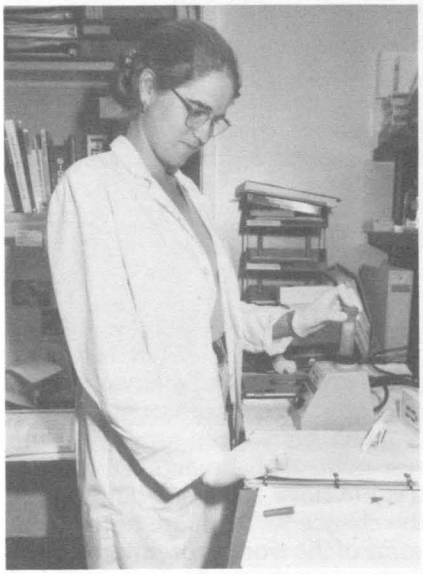
The genetic study of XLPRA relies on the informative pedigree bred and maintained by this laboratory. The disease is detected clinically in affected individuals within their first two years, with blindness occurring in most individuals between five and six years of age.

My project involved culturing, preparing, and labeling human cDNA probes for cross-species Southern blotting. I used the G6PD probe from the long arm of the human X chromosome. Previous work by Dr. Hershfield had used probes from the short arm of the same chromosome. The G6PD probe was employed to examine the DNA from key dogs throughout the pedigree using multiple restriction enzyme digests. After finding a possible RFLP using the restriction enzyme Dra I, DNA samples from multiple dogs from important branches of the pedigree were digested with this enzyme and hybridized with G6PD to confirm the existence of the RFLP. Linkage between the RFLP and XLPRA was examined by testing the inheritance patterns of the RFLP compared with XLPRA within a nuclear family (consisting of father, mother, and multiple male and female offspring) from the pedigree. The laboratory will continue to examine the pedigree using this RFLP, with the ultimate goal of determining the genetic location of the XLPRA defect through RFLP linkage analysis.

Participating in the Leadership Program was an extremely rewarding experience for me. The lab I worked in provided me with a technically and intellectually challenging summer. The combination of research, educational, and social encounters that were an integral part of this program have clarified my career options. I would highly recommend the program to anyone who is even remotely considering an academic veterinary career. I would also like to thank Linda Griswold and Carol Miltenberger for coordinating the many events of the past summer, and the program organizers for granting me this unique opportunity.

"Linkage between the RFLP and XLPRA was examined by testing the inheritance patterns of the RFLP compared with XLPRA within a nuclear family (consisting of father, mother, and multiple male and female offspring) from the pedigree."

Mary Thompson - University of Sydney



I am midway through my fourth year at the University of Sydney and am looking forward to my fifth and final year living in Camden, New South Wales where the University's large animal clinic and farms are located. I have always had a great interest in clinical veterinary medicine and surgery, but recognizing the fact that I will complete my veterinary degree at the age of 22, I have also maintained an interest in broadening my experience, especially in research. Despite an interest in biomedical research, I never had the opportunity to indulge it, however. When I saw a poster advertising the Cornell Leadership Program for Veterinary Students in my early years at Sydney University, I was determined that I would attend the program and use the opportunity to gain information, guidance and experience which would enable me to structure my career in an informed manner.

As a Mellon Foundation Fellow, I had the opportunity to work with Dr. Edward Pearce in the Department of Microbiology, Immunology and Parasitology. The focus of research in this laboratory is the trematode parasite, *Schistosoma mansoni*, which causes great morbidity and mortality in humans in many parts of the developing world.

Infection with schistosomes is initiated when the aquatic infective stage, the cercaria, penetrates the skin of its definitive host. During the 24 h following invasion, cercariae transform into schistosomula. As they do so they undergo extensive surface adaptations which allow them to live within their host. Transformation can be induced artificially in vitro. After 24-48 h in the skin, schistosomula enter the portal venous blood and begin a 2-3 wk migration to the liver, their preferred site of parasitism. The objective of my research was to identify molecules expressed on the surface of the parasite during this initial period of adaptation to the host when the parasite acclimatizes to its new environment.

My summer project focused specifically on the expression of surface membrane tyrosine kinases. Tyrosine-kinase-linked receptors have been well characterized. Many have been identified and found to be involved in a number of important cellular functions including cell growth, development and differentiation, all of which may be important to a changing and adapting parasite such as S. *mansoni*. As with many receptors, binding of the complementary ligand activates a chain of events leading to DNA, RNA, or protein synthesis or cell division. The surface of the schistosome is unusual for a large multicellular organism insofar as it consists of a lipid bilayer, making the localization of tyrosine kinases to this interface a possibility.

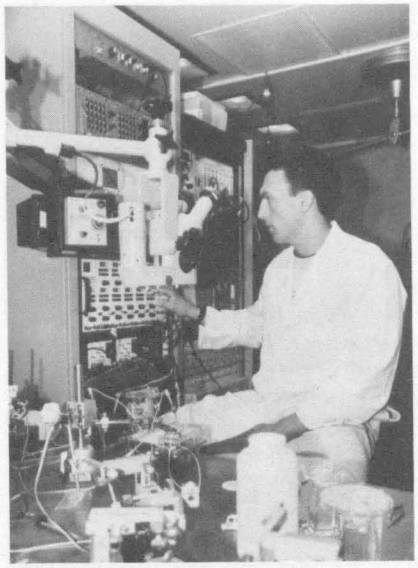
In order to specifically examine surface proteins of schistosomula, it was necessary to label them in a way that would allow them to be distinguished from non-surface proteins. To do this I chose to biotinylate intact schistosomula using a form of biotin that could not penetrate the surface membrane. In this procedure, biotin becomes covalently attached to surface proteins, which can subsequently be identified and/or isolated using strepatavidin, a protein which specifically binds to biotin.

With the aim of identifying kinases exposed at the surface membrane of schistosomula, I performed in vitro kinase assays on detergent extracted surface-biotinylated proteins precipitated on streptavidin agarose beads. Surface proteins of 3 h and 24 h old schistosomula derived from in vitro-transformed cercariae were utilized. Following incubation of surface-biotinylated proteins with ^{32}P-ATP, the proteins were electrophoresed on a polyacrylamide gel. The gel was then dried and exposed to photographic film. A control sample of protein in which an excess of unlabelled ATP was added prior to the addition of ^{32}P labelled ATP was prepared to discount the possibility of non-specific interaction of ^{32}P-ATP with surface proteins that were not kinases. Using this technique, I was able to demonstrate that whereas 3 h old schistosomula do not express surface kinases, 24 h schistosomula express several. Many more experiments, including phospho-amino acid analyses will need to be performed in order to confirm that the kinases present on the surface of schistosomula are tyrosine kinases. A Western blot of surface molecules using a monoclonal antibody specific for anti-phosphotyrosine is currently underway with the aim of providing further supporting evidence.

The major value of the Leadership Program was that it provided an excellent opportunity to consider and sample future career options, both in research and as a practicing veterinary surgeon. For someone like myself, who lacked research experience, it was interesting to work in a laboratory atmosphere where colleagues were eager to give advice and guidance. To this end I must thank Ed Pearce, Stephen Davies, Beth Sabin, Joao Pedras, Susan Leonard and David Fitzhugh for their help throughout the summer.

"... whereas 3 h old schistosomula do not express surface kinases, 24 h schistosomula express several."

Oliver Turner - Liverpool University



My quest to become a veterinarian began when I entered the University of Liverpool, England, in 1989. After three years of preclinical science, I elected to augment my developing interest in scientific research by pursuing an intercalated B.Sc. degree in Physiology. My dissertation, sponsored by the Wellcome Trust, involved quantifying developmental and gestational changes in the levels of phosphoethanolamine and taurine in murine uterine smooth muscle, compared to skeletal and cardiac muscle, using high performance liquid chromatography. This virginal experience into research left a good impression, so when the chance to apply for the Leadership Program arose, I jumped.

Wellcome continued its generous support of my intellectual explorations which in this instance were pursued in the Department of Physiology. I was privileged to work under the guidance of Dr. Robert Gilmour Jr. who is currently investigating several cardiac muscle diseases using electrophysiological techniques. They include Chagas disease, the development of a model for SIDS, and the application of nonlinear dynamics to cardiac dysrhythmias. It was in the latter field that I became most concerned, looking into the behaviour of the electrical restitution curve.

The electrical restitution curve is a standardized method for investigating the effect of transient changes in beat to beat interval on the electrophysiology of the myocardium. The restitution curve is generated by plotting the duration of the cellular action potential (APD) as a function of the stimulus coupling interval. Most often, a steep ascending slope of restitution is followed by a plateau phase - APD increasing almost monoexponentially with coupling interval. But, under certain conditions this phenomenon has been shown to take on a biphasic form, rising more steeply than normal, to a maximum, and exhibiting a depressed plateau phase. The mechanism for biphasic restitution is unknown. My work involved an examining of whether elevated extracellular potassium could produce this effect.

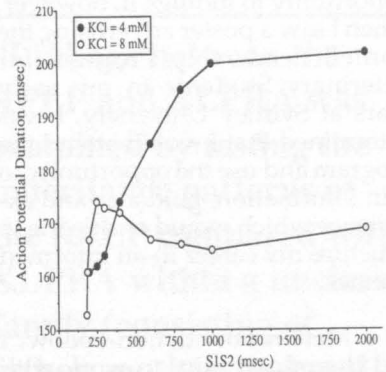
Canine ventricular epicardial strips were mounted in a tissue bath perfused with oxygenated Tyrodes solution. A pulse train of 300ms, 500ms or 800ms coupling intervals drove the muscle, and premature stimuli were applied at variable intervals after every 10th to 20th driving stimulus. Action potentials were recorded using conventional microelectrode techniques. Normal Tyrodes, with 4mM potassium, was replaced by 8mM, 10mM and 12mM solutions. Action potentials were displayed on an oscilloscope and photographed. Measurement of action potential amplitude (APA), APD50 and APD90 were obtained. Restitution curves were then generated.

At the time of writing, it appears that a biphasic response is generated by a hyperkalemic environment, particularly in the experiments with the 500ms and 800ms pulse trains and at 10mM potassium Tyrodes. The phenomena thus appears to exhibit both a cycle length and extracellular potassium concentration dependence.

The mechanism for this result may involve augmentation of calcium influx (I_{Ca}) in response to the premature depolarizations, with a concurrent damping of changes in potassium efflux (I_K) secondary to the hyperkalemia. Given that hyperkalemia is an important consequence of acute myocardial ischaemia, and biphasic restitution has been shown to underlie complex dynamical behaviour, these results may pertain to the development of ventricular tachyarrhythmias during acute ischemia.

My time at Cornell has revealed exciting new dimensions to the veterinary profession and many possibilities for postgraduate training therein. Not only have I seen some of the top research establishments of this country, but have also been honored by the chance to 'compare notes' with some of the world's premiere veterinary researchers. Along with this, I have spent my summer with a luscious group of geographically and culturally diverse, but like minded students, with whom I will have many long-standing memories and even longer friendships.

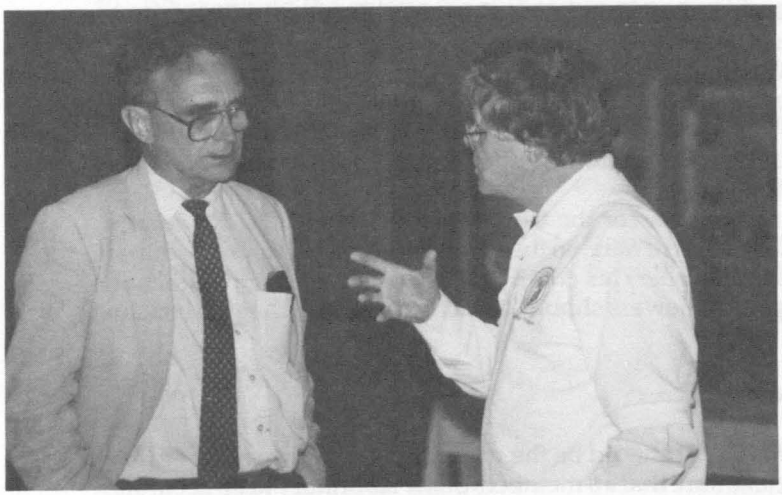
I would like to heartily thank Dr. Gilmour, Weiping Han and Mari Watanabe for giving me a brief insight into a fascinating field of physiology. I go back to Liverpool to finish my quest with heightened passion.



Relationship between the stimulus coupling interval (S1S2) and action potential duration (i.e. the restitution of action potential duration) in canine ventricular myocardium during *in vitro* superfusion with normokalemic (KCl = 4 mM; filled circles) and hyperkalemic (KCl = 8 mM; unfilled circles) Tyrode solution. Hyperkalemia converted monotonic restitution to biphasic restitution.

Stacy Pritt and Larissa Bowman participated in the Leadership Discussion.

Les Gabor, Amanda Gaskin, Chris Mariani, Jeff Nunez and Maria Lara enjoying the Welcoming BBQ.



Dr. Douglas McGregor and Dean David Fraser exchanging thoughts on the Leadership Discussion.

Ethics Committee Report

Committee: Mr. John MacGregor, Ms. Sonia Mumford, Ms. Maria Lara-Tejero, Ms. Amanda Gaskin, and Ms. Stacy Pritt.

Summary of Discussion

Question - *What constitutes scientific misconduct? Is the current NSF definition adequate?*
Group Consensus - Scientific misconduct should consist solely of plagiarism and fraud, with fraud defined as the reporting of falsified data and/or intentionally misrepresented results. The last part of the NSF definition was considered to be too vague in this connection. Scientists should subscribe to generally accepted methods of data analysis. When appropriate, methods that decrease biased results (e.g., double blind testing) should be used. But the use of unconventional methods should not be considered scientific misconduct provided the methods and reasons for choosing those methods are detailed in the publication of the results. Misconduct exclusive of plagiarism or fraud should be dealt with in another arena - e.g. an offense such as sexual harassment should be dealt with as such by whatever university/institutional, local, state or federal statutes apply.

Question - *Does the present system motivate scientists to commit fraud? Can the system be improved? How?*
Group Consensus - Some members of the group thought that the prevailing system was good because it motivates scientists to be productive and not become complacent. But, other members of the group expressed the view that revision of tenure and/or grant funding mechanisms was in order, so that the "publish or perish" frame of mind could be eliminated or at least ameliorated. These individuals would prefer a system that included start-up grants for all those awarded positions in research institutions and ongoing funding based on a review of their previous five years of work.

Question - *Can scientists police themselves in cases of scientific fraud? What should be the roles of the media and the federal government?*
Group Consensus - A scientific panel convened by the institution should have the first opportunity to deal with allegations of misconduct. The committee should consist of non-biased, non-affiliated, yet knowledgeable scientists. If the results of their inquiry are not satisfactory to the person making allegations, the funding institution should convene an appeals panel. If the results of the panel inquiry are not satisfactory, or either body has found sufficient evidence of fraud, the case should be referred to the appropriate judicial body.

Question - *What is meant by critically reviewing a manuscript for publication? Does it require examination of raw data?*
Group Consensus - This question was not addressed to any great extent in the discussion. However, the view was expressed that reviews should be conducted by an informed panel of scientists. Conclusions should follow logically from the data. Reviewers need not examine the raw data, but should be allowed access to the data should they desire it.

Question - *Whose responsibility is it to confirm that a scientific paper is valid? Is it the responsibility of the author? the peer reviewers? or the test of time?*
Group Consensus - The authors are responsible for the truthfulness of their data. Reviewers might not be able to detect a skillful fabrication of data, so the system rests solely upon the honor of the authors. Each author must ensure that her/his contribution to the paper is truthful and accurate. Additionally, authors and peer reviewers should collectively ensure that the conclusions follow logically from the data.

Suggestions for Improving the System:

1. Scientists should keep good notebooks. It should be the responsibility of the senior author and the head of the laboratory to make sure that all members of the laboratory keep accurate, up-to-date notebooks, as an ongoing practice and particularly when publication of the data is contemplated.

2. All federally-funded research institutions should have protocols in place to deal with allegations of scientific misconduct. At a minimum, the protocols should call for the convening of a panel of non-biased, non-affiliated scientists in similar fields to investigate allegations of plagiarism or fraud.

3. There should be clearly delineated federal statues regarding scientific fraud with clearly specified penalties. Other funding institution also should have protocols for dealing with fraud, and anything found to be fraudulent should be turned over to the government for further action.

4. The scientific community should improve its relationship with the popular media and the public. This might involve media training courses and presentation of results in terms that the general public can understand, to the extent possible. This would allow scientists to become less defensive in their dealings with the media and also would engender a more positive public attitude towards science and scientists.

5. During fraud inquiries, scientists should not become defensive and should allow the system to work. The funding institution has the right — and in some instances the obligation — to investigate how its awards are used without being accused of politicizing or manipulating science. Federal funding institutions have a responsibility to the tax paying public to make sure that their tax dollars are being spent honestly, and scientists must accept this scrutiny.

Additional Questions Emerging from the Discussion:

6. When does scientific misconduct become a criminal act?

7. What is the role of the host institution in the investigation of scientific misconduct? Can it be objective?

8. What impact do the credentials of the "whistle blower" have on the investigation of scientific misconduct? What impact should it have?

9. To what degree should one trust one's colleagues when allegations of fraud are made against them?

10. Should there be due process in cases of scientific misconduct? If so, what are the components of a fair and effective system for dealing with such cases?

11. Is it more important to protect the reputations of scientists than have truth in science?

12. What should the criteria for authorship of a scientific paper be?

13. What responsibility does each co-author have to make sure that a scientific paper is valid?

14. What role if any should the Office of Scientific Integrity have in the scientific community?

15. Is there a common standard of how research should be conducted?

16. Are the precedents of this "trial" i.e. Congressional intervention, forensic evidence produced and made available to the Office of Scientific Integrity (but not subject to independent analysis) dangerous?

Acknowledgments

The program organizers wish to thank the Fundación Purina, the Richard King Mellon Foundation, the Merck Foundation, the Wellcome Trust, and the Robert W. Woodruff Foundation for their generosity and vision in sponsoring this program. We also thank the Auxiliary of the New York State Veterinary Medical Society for providing several program prizes. Special thanks are extended to Ms. Maureen Mylander and Dr. T.J. Sexton for arranging splendid programs at the NIH and USDA, respectively. Dean Robert D. Phemister and Professor Ari van Tienhoven arranged stimulating discussions of leadership and biomedical ethics as they have done in earlier years. Their participation, and that of Professor Barbara A. Baird, Ms. Susan E. Baptiste, Dr. Peter M. Biggs, Professor Richard A. Cerione, Dr. Patricia M. Chisholm, Dr. Robert E. Donahue, Professor Brian R.H. Farrow, Dean David R. Fraser, Dr. Michelle L. Haven, Dr. Gerard J. Hickey, Professor Francis A. Kallfelz, Professor Robert M. Lewis, Professor James N. MacLeod, Mr. Thomas J. McGiff, Professor Edward J. Pearce, Dr. Larry J. Thompson, and Dr. Jeffrey L. Ward contributed in important ways to the success of the program. Their enthusiasm and wisdom will be long remembered by the participating students and the program organizers and staff.

Document design by Linda A. Griswold.
Photographs by George W. Cannon, Reinhard K. Straubinger, and Carol D. Miltenberger.