



The James A. Baker

INSTITUTE FOR ANIMAL HEALTH

COLLEGE OF VETERINARY MEDICINE • CORNELL UNIVERSITY

Annual Report, 1997, Volume 47

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The James A. Baker
INSTITUTE FOR ANIMAL HEALTH

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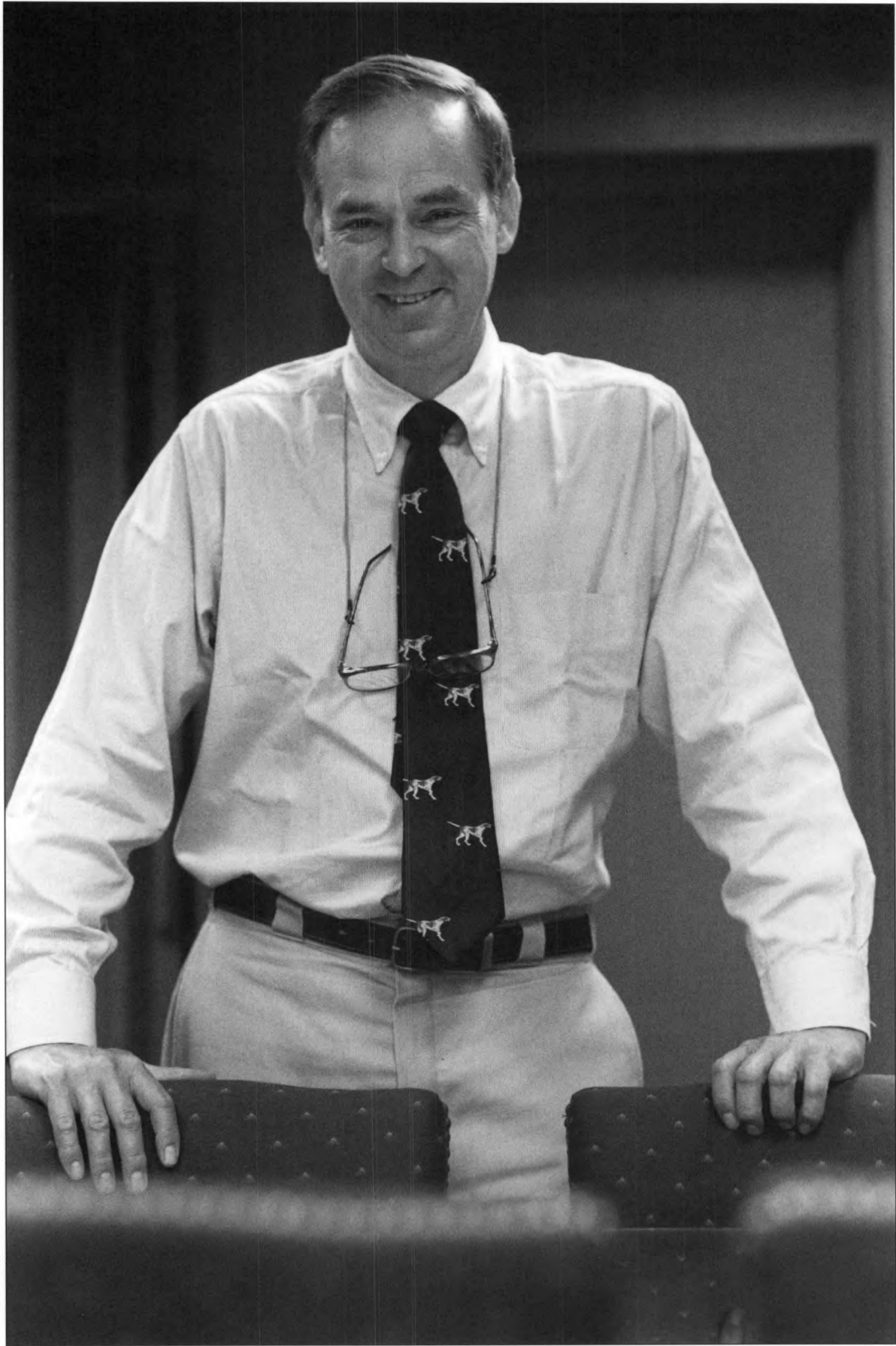
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A Message from the Director

THE YEAR 1997 brought many successes to the Baker Institute, but also significant change. Our programs in animal health research continued to grow, fueled by the imagination and hard work of the Institute's scientific staff. Research activity reached an all-time high in both of our major program areas, infectious diseases and immunology, and genetics and development.

In July we hosted the second meeting in our series of Baker Institute scientific conferences. Organized by Institute scientists Drs. Gus Aguirre, Greg Acland, and Kunal Ray, the International Workshop on Canine Genetics brought the world's leading researchers in dog genetics to Cornell for three days of presentations of research results and lively discussion about future directions for investigations of canine genetics. More details about this workshop can be found elsewhere in this report.

In 1997 the Institute's new Laboratory of Cell and Molecular Biology gained a third major instrument, a flow cytometer, which is now in use along with the genetic analyzer and phosphor-imager already in place. Acquisition of this important piece of equipment was made possible by a generous grant from the Mrs. Cheever Porter Foundation. Flow cytometry has become an essential technique for analyzing complex mixtures of white blood cells. As an example, immunologists use flow cytometry to determine the CD4:CD8 ratio, a relative measurement of two types of T-lymphocytes used to evaluate the immune systems of AIDS patients.

Institute scientists are employing flow cytometry for a variety of applications, including studies of canine parvovirus infections and immunity in the equine reproductive tract. We are very grateful for the financial support that has allowed us to equip the Baker Institute with this sophisticated but essential scientific instrument.

By far the most important events of the past year were the retirements of the Institute's two most senior scientists—Professors L. E. “Skip” Carmichael and Max Appel, which the Institute marked with a small celebration in September of 1997. Dr. Carmichael, the John M. Olin Professor of Virology, came to the Institute in 1955 to begin postgraduate research under the tutelage of the Institute's founding director, James Andrew Baker. Fresh from his veterinary studies at the University of California at Davis, Dr. Carmichael embarked on an illustrious career in veterinary infectious disease research that has spanned more than four decades. Dr. Appel, who came to the Institute in 1964 to study with Dr. Carmichael, has had an equally successful run. A retrospective of Dr. Carmichael's career appeared in last year's annual report; this year Dr. Appel also takes a look back.

Filling the void created by the retirements of Drs. Appel and Carmichael will not be easy. Fortunately for us, both men remain active in the Institute as emeritus faculty. In the long run, how we adapt to these changes will determine in large measure our future success. The Institute is committed to the continual reinterpretation of its historic focus in infectious disease research in the context of the problems that confront us now, and to

those that loom over the horizon in the coming century.

We are also dedicated to the continued strengthening of our primary mission of basic and applied veterinary research. There are few institutions in the world with a tradition and record like ours. A major challenge for the Institute is to redefine our mission in light of the changes that are occurring in society. The initiation of the Institute's new program in genetic medicine is an example of our response to such changes.

A second major challenge for us is to continue to improve and expand our facilities for the growing numbers of researchers who have made the Institute their scientific home. We have begun to address the structural and fiscal constraints to a modest but necessary expansion, and we are confident that we can continue to grow while maintaining the special nature that has characterized this foremost veterinary research institute for almost half a century.

The Baker Institute is blessed with a talented staff and bright and enthusiastic students dedicated to the solution of important problems in veterinary medicine through research. It has always been a privilege for me to work in this most stimulating environment, and I take great pleasure in bringing you this report of our activities of the past year. Good reading!

—Douglas F. Antczak



Laurie Lychalk

Staff of the Baker Institute

ADMINISTRATION

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Director: B.A., Cornell U.; V.M.D., U. of Pennsylvania; Ph.D., Cambridge U.

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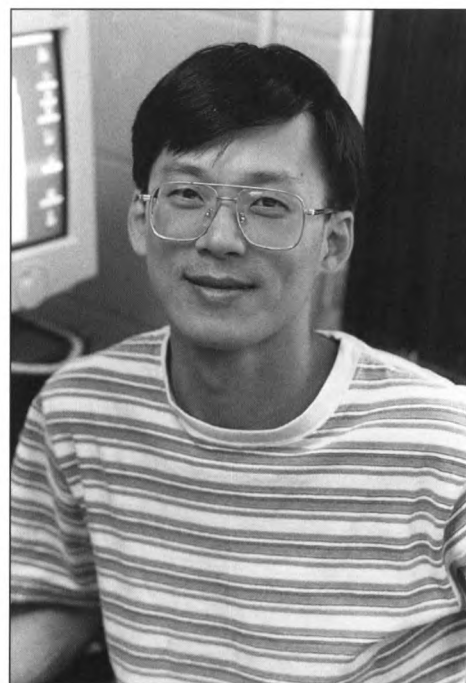
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Chih-Tung Chen

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MAINTENANCE

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Facilities coordinator

Richard E. Daniels

Maintenance mechanic

Hubert Dorn, Jr.

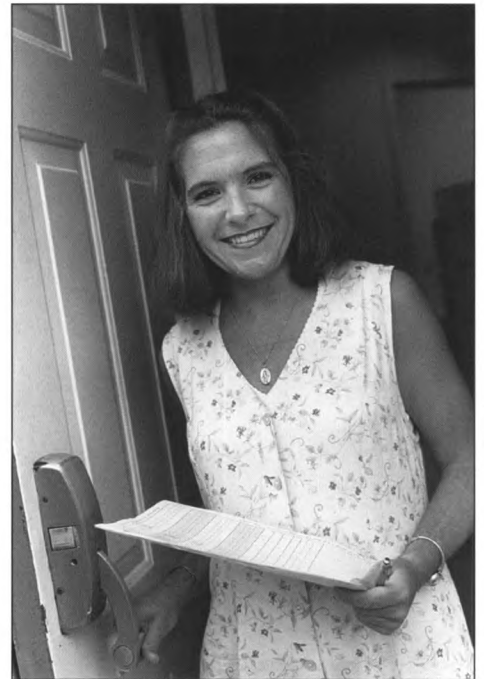
Maintenance mechanic

Russell F. Haus

Maintenance mechanic

Michael Wright

Laboratory attendant



Gail Sullivan (top) and Denise Markley-Heichel (bottom)



Perspectives

AS YOU WILL SEE as you read this report, the Institute is thriving. The research programs are growing, new investigations are continuing to evolve, and the success of those investigations has been impressive. With success, however, there is sometimes a risk of developing a sense of complacency. We have to build on this momentum. Now, as the Institute nears its fiftieth anniversary in the year 2000, is the time when we have to work tirelessly to increase its visibility, to attract more funding, to recruit new minds, and to ask longtime staff members for new ideas.

Any scientific institute's success is based on a special mix of the stability that experienced members offer and the energy and innovation usually associated with younger scientists. These devoted people are the basis of the success of the Baker Institute. It is the Institute's job to identify and resolve emerging threats to animal health. We who support the Institute must do our share to help them secure the funding and the facilities that these efforts require.

Just as the continued vitality of the Institute depends on renewal among faculty and staff, it depends on renewal among its supporters. We look to our new friends to contribute to the success of the Institute in the same manner as in earlier generations. In addition, we look forward to the continued help of our old friends. Without their support, the Institute would never have achieved the prominence it has today, among both the scientific and the animal-owning communities.

—Henry J. Travis, D.V.M.
Advisory Council Chairman

Advisory Council

Henry J. Travis, D.V.M., Chairman

General Practitioner, Huntington, New York

Stephen H. Blose, V.M.D., Ph.D.

Bio-Rad Laboratories, Hercules, California

Sarah R. Bogdanovitch

Lake Clear, New York

Albert C. Bostwick, Jr.

Aiken, South Carolina

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College of Veterinary Medicine, North
Carolina State University at Raleigh

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Chief Scientific Officer, The Foundation
Fighting Blindness, Hunt Valley, Maryland

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General Practitioner, Deer Park, New York

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Medicine, University of Pennsylvania

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M.D. (hc)**

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Microbiology, and Immunology, School of
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Gene M. Pranzo

President, The Dorothy Russell Havemeyer
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Andrew G. C. Sage II

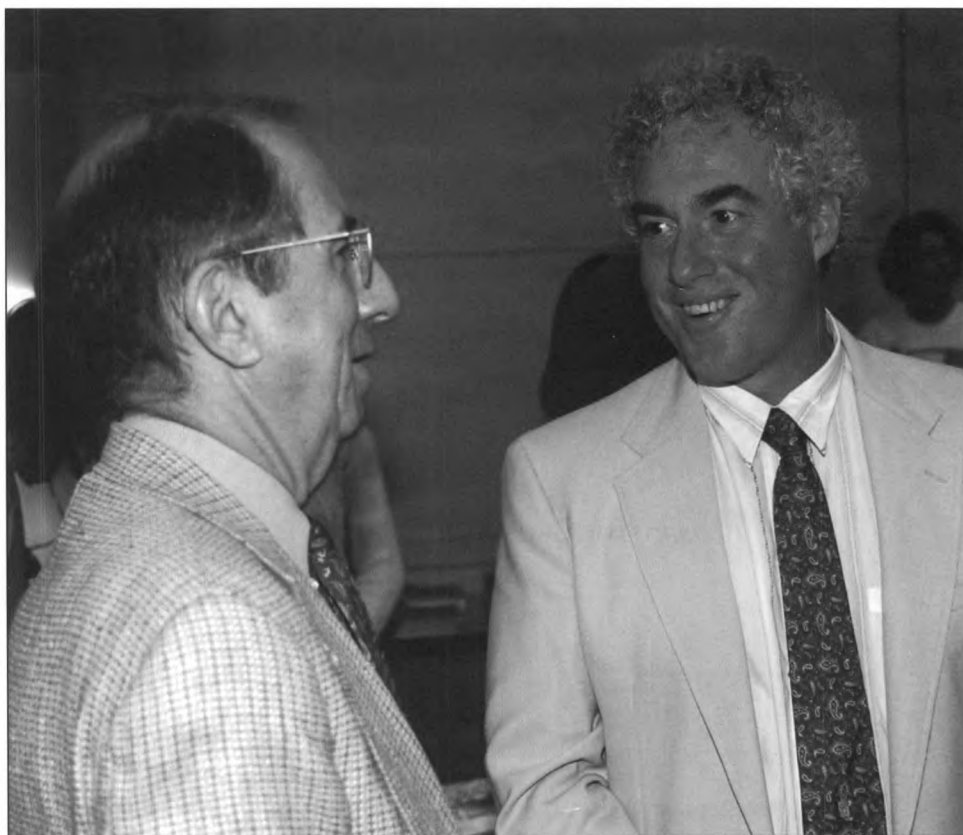
Glen Cove, New York

Robert E. Shope, M.D.

Department of Pathology, University of Texas
Medical Branch, Galveston

Judith Wilpon

Locust Valley, New York



Advisory Council members Gene Pranzo and Hank Travis

FORMER ADVISORY COUNCIL MEMBERS

William C. Beck, M.D., F.A.C.S.

1982-1994

Dorothy R. Donnelley

1980-1992

Chairwoman, 1982-1988

Strachan Donnelley, Ph.D.

1990-1995

G. Watts Humphrey, Jr.

1982-1989

Richard M. Johnson

1977-1989

Joseph W. Jones

1982-1996

Patricia Kaneb

1989-1996

John A. Lafore, Jr.

1978-1984

Gary Lee

1977-1986

Irwin H. Lepow, M.D.

1978

John M. Olin

1977-1982

Niel W. Pieper, D.V.M.

1977-1993

William Rockefeller

1979-1990

Chairman, 1989-1990

Frances G. Scaife

1978-1988

Robert Winthrop

1982-1990

Robert Winthrop II

1984-1994

Recognitions

We departed from tradition this year to confer the North and Founders' Awards on two of our own faculty members, **Max Appel** and **Leland "Skip" Carmichael**. In considering the purpose of these awards—to recognize service to dogs and to veterinary medicine—we concluded that no one fit the criteria better than these two veterans of the infectious disease wars. Dr. Appel, whose retrospective appears later in this report, retired and was granted emeritus status in October, 33 years after coming to the Institute as the first graduate student of then-assistant professor Leland Carmichael. Dr. Carmichael, who had come to the Institute as Dr. Baker's graduate student, made the transition to emeritus status at year's end. His look back at his 41 years at the Institute was featured in last year's annual report.

Between them, Dr. Carmichael and Dr. Appel have made major advances against most of the viral and bacterial diseases known to pose a significant health threat to dogs. Those diseases include infectious hepatitis, herpesvirus, brucellosis, kennel cough, coronavirus, minute virus of canines, Lyme disease, canine distemper—which has also cropped up repeatedly in wildlife species—and the infamous parvovirus, whose catastrophic spread they halted with remarkable dispatch.

In recognition of these and other contributions to the safety and health of the canine pet population, we honored them: Dr. Appel with the Arthur F. North Canine Service Award, and Dr. Carmichael with the Founders' Award. The award presentations were made during a weekend-long "symposium," which was conducted in the convivial sense Plato intended, following the September meeting of the Advisory Council. More than 200 friends, colleagues,



Mary Margaret and Skip Carmichael greet friends at a luncheon honoring Drs. Carmichael and Appel

and former students gathered in Ithaca to reminisce and toast these two men whose accomplishments account for much of the Institute's renown.

THE ARTHUR F. NORTH, JR. CANINE SERVICE AWARD

Arthur F. North, D.V.M. '35, was a skilled and innovative practitioner and an enthusiastic friend of the Baker Institute. The North Award recognizes those whose con-

tributions to canine health and well-being reflect his spirit of concern for all dogs.

THE FOUNDERS' AWARD

The Founders' Award was established in celebration of the Institute's 40th anniversary in 1990. This recognition is given annually to a veterinarian whose contributions to the Institute and to his or her profession exemplify our founders' commitment to the advancement of veterinary medicine.

AWARD RECIPIENTS

North Award

- 1982 Adelaide Riggs
- 1983 The American Kennel Club
- 1984 Priscilla Maxwell Endicott
- 1985 The Marilyn M. Simpson Charitable Trusts
- 1986 Frances Rowles Van Brunt
- 1987 The Geraldine R. Dodge Foundation
- 1988 Atherton Bristol
- 1989 Jacqueline Lindsay
- 1990 Dorothy Donnelley
- 1991 Robert Winthrop
- 1992 Eleanor Gillis
- 1993 Albert C. Bostwick, Jr.

- 1994 Dolly B. Trauner
- 1995 Barbara J. Hartsig
- 1996 Robert W. Kirk, D.V.M. '46
- 1997 Max J. G. Appel, D.V.M., Ph.D. '67

Founders' Award

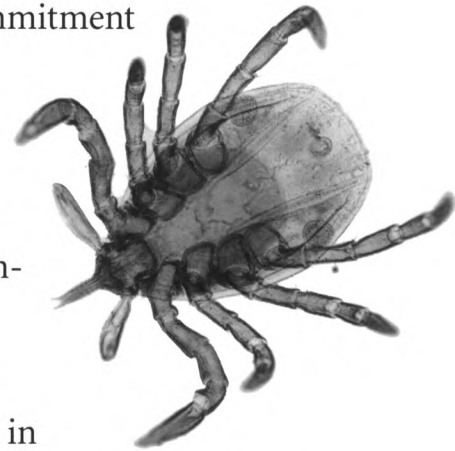
- 1990 Charles Fletcher, D.V.M. '33
- 1991 Du Bois Jenkins, D.V.M. '43
- 1992 Niel Pieper, D.V.M. '32
- 1993 Harold Kopp, D.V.M. '42
- 1994 G. Clayton Dudley, D.V.M. '64
- 1995 John A. Ward, D.V.M. '36
- 1996 Robert E. Clark, D.V.M. '52
- 1997 Leland E. Carmichael, D.V.M., Ph.D. '59



Infectious Diseases and Immunology

SINCE ITS FOUNDING IN 1950 as the Veterinary Virus Research Institute, the Baker Institute has maintained outstanding programs in basic and applied research on infectious diseases of animals. In particular, the Institute has led the world in developing practical means to prevent and control infectious diseases of dogs. Our commitment to this important area remains firm.

Over the past 20 years we have witnessed the amazing ability of infectious agents to adapt to new hosts and environments, and to elude and evade host immune responses and chemotherapy through genetic mutation. Such changes in pathogenesis have decreased the usefulness of the traditional antibiotics and vaccines that have long been successful in controlling many infectious diseases. The continuing threats to animal and human health from infectious agents will require new strategies and approaches to this age-old problem.



Opposite: Dr. Barbara Butcher using the flow cytometer purchased with a grant from the Mrs. Cheever Porter Foundation.

Right: A nymph of the tick species *Ixodes scapularis*. Such ticks can transmit the bacterium that causes Lyme disease.

Fortunately, new knowledge of cell and molecular biology has created tremendous opportunities for advances in the continuing battle against micro-organisms. Baker Institute scientists have added these tools to their traditional armamentarium of skills in virology, bacteriology, parasitology, and immunology. The following reports describe the Institute's current projects aimed at improving methods for the control of infectious diseases. The studies range from the very practical—for the problems of today—to the theoretical, so that we may have the understanding to solve the new problems that surely lie just beyond the horizon.

Hadley C. Stephenson Laboratory for the Study of Canine Diseases

As in humans, the hallmark of Lyme disease in dogs is recurrent acute arthritis in joints such as the shoulder, elbow, and knee. The clinical signs of lameness develop months after infection with the bacterial agent, *Borrelia burgdorferi*. The onset of the arthritis is sudden, and the number of leukocytes in the synovial fluid in the joints rises dramatically during these episodes.

Over the last few years we have focused on the role of the chemokine interleukin (IL)-8 in the acute phase of Lyme arthritis in dogs. We have now expressed the IL-8 protein *in vitro*, produced polyclonal antibodies against this chemokine, and characterized its up-regulation prior to the onset of clinical signs. We have shown that this up-regulation, or increase in production of IL-8 messenger RNA, coincides with the migration of polymorphonuclear neutrophils, a subset of leukocytes that can account for 95 percent of the cells found in Lyme-arthritic joints. However, the cause of the up- and subsequent down-regulation of IL-8 is still unknown, and our present goal is to define the sequence of events that leads to tissue inflammation and its resolution. To this end we are also focusing on two other potentially important factors, IL-1 α and IL-10.

IL-1 α is a potent stimulus for IL-8 production and is one of the cytokines known to initiate and regulate inflammation. Using reverse-transcription polymerase chain reaction, postdoctoral associate Alix Straubinger found that IL-1 α messenger RNA (mRNA) was up-regulated in inflamed tissues of dogs with Lyme arthritis. Dr. Straubinger also cloned and determined the DNA sequence of canine IL-1 α

as well as the complete coding sequence of feline IL-1 α .

The other factor we are investigating, IL-10, is known for its inflammation-inhibiting properties. We are studying its effect by co-culturing recombinant human IL-10 (rhIL-10) and *B. burgdorferi* with canine synovial tissue from uninfected dogs. In a series of experiments, we added rhIL-10 to the culture system at varying time points. We found that the production of proinflammatory cytokines was inhibited when rhIL-10 was added to the culture system. Inhibition was greatest when rhIL-10 was added before or simultaneous to exposure to *B. burgdorferi*. Even when added later, however, rhIL-10 reduced the expression of all proinflammatory cytokines.

These results might help us to understand why the acute arthritis in dogs and

humans is transient and self-limiting during the early stage of Lyme disease. It had been thought that the polymorphonuclear neutrophils (PMNs) accumulated in the joint to eliminate or at least reduce the number of bacteria in the tissue. However, we found that the enzymes and molecules released by PMNs are not specifically directed against *B. burgdorferi*. Furthermore, these substances damage the surrounding synovial tissue. Therefore it is possible that host-derived immunoregulatory factors such as IL-10 are released into the system to minimize further tissue damage. In future experiments we will test to see whether this cytokine is involved in the pathogenesis of acute Lyme arthritis.

We continue to study the efficacy of antibiotic regimens against Lyme disease; we treated dogs orally with doxycycline or azithromycin or intravenously with ceftriaxone, a synthetic, third-generation penicillin. We also studied the effect of oral corticosteroid treatment on previously infected dogs. None of the antibiotic-treated dogs that received corticosteroids showed clinical signs of Lyme disease. However, corticosteroid treatment in untreated dogs reactivated acute arthritis quickly, an indication that subclinical Lyme disease might cause complications if treated improperly or if the disease remains undiagnosed.

Our laboratory also underwent an important transition in personnel in 1997. As noted elsewhere in this report, Dr. Appel retired in October and was awarded emeritus status. In contrast, I finished my Ph.D. earlier in the year and have stayed on as a research faculty member. Our titles have changed, but our efforts to understand the workings of canine Lyme disease continue.

—Reinhard K. Straubinger

We want to understand why the acute arthritis in dogs and humans is transient and self-limiting during the early stage of Lyme disease.



Reinhard Straubinger

Albert C. Bostwick Laboratory of Molecular Biology

Twenty years after it suddenly appeared as a new pathogen of dogs, spreading worldwide in six months, canine parvovirus (CPV) still causes significant clinical disease in dogs. We are interested in the relationship between this emergent virus and the long-recognized feline panleukopenia virus (FPV). Our studies are aimed at defining where CPV came from and how it gained the new properties that allowed it to become a successful canine pathogen.

Our previous studies have focused on examining the differences between the canine and feline parvoviruses to determine which of those differences controls the ability of the virus to infect dogs or cats, or their cells in culture. The results clearly showed that the host range differences between those viruses are determined by sequence differences in the virus coat protein gene and that the specific changes were on the surface of the viral coat, or capsid.

We have now turned our attention to the cells of the host animals—dogs and cats—and the differences between them that determine their susceptibility to the canine and feline viruses. The problem is a complex one that concerns a critical step in the interaction between the virus and its host cell, and how that interaction has been altered by the mutations that have occurred in the canine virus. All animal viruses replicate inside cells; to do so they have to find a way to pass from

the outside of the cell to its interior. Once there, they also have to deliver their infectious materials, including the viral genome, into the correct cellular compartment for replication. It appears that the infection of canine cells by FPV is blocked at an early stage, after the virus binds to the surface of the cell but before the DNA is delivered into the nucleus, where it can replicate. In contrast, CPV passes through the same step in uptake and successfully infects the cell.

Little detail is known about the pathway that the viruses use to infect the cell successfully, and so in some initial studies we are defining aspects of the pathway that CPV uses to enter the cell. John Parker is examining the process of early entry and uptake using mutants of molecules called dynamin, rab5, and rab7, which control the formation and

trafficking of membrane vesicles during uptake. These studies will also involve using various microscopic methods to show where the virus particles go in the cell during the process of infection.

Cat and dog cells differ in the genetic properties that determine their susceptibility to the different viruses. Dai Wang has analyzed hybrid feline-mouse cells containing defined numbers of feline chromosomes to show that the susceptibility to FPV is a property of feline chromosome C2. Now he is using a variety of approaches to clone individual feline genes that control virus susceptibility.

In other studies Wen Yuan and Wendy Weichert have been examining the structures and functions of the CPV capsid for changes that occur during the process of infection. In those studies Ms. Weichert has been examining the role of proteolytic cleavage of the capsid protein in cell infection, while Mr. Yuan has been examining how the individual proteins assemble within the cell and package the viral DNA.

These studies will lead us to a complete understanding of the replication processes of these parvoviruses that cause significant and severe disease in dogs and cats. They should allow us to devise better strategies for disease control and to understand at a basic level how canine parvovirus was able to emerge as a new virus in dogs.

— Colin R. Parrish

We are studying the differences between dog and cat cells that determine their susceptibility to the canine and feline parvoviruses.



Colin Parrish and Wen Yuan



Immunology Laboratory

Our work during this decade has focused on the role of immunoglobulin E (IgE) in rejection of the parasitic nematode *Trichinella spiralis* from the intestine. IgE is known to be the principal cause of allergic reactions to antigens such as pollens or danders, and its involvement with mast cells during this process has been well studied. In contrast, little is known of IgE's presumed benefit. While IgE production has been known to be powerfully stimulated by intestinal nematode infections, demonstrating that IgE was important in protecting the host proved an elusive task. Not only have the underlying processes leading to the expulsion of larval and adult worms from the intestine been historically difficult to unravel, but recent evidence has shown that the parasite effectively changes its coating of antigens—which are the targets of antibody, including IgE—as it molts from the larval to the adult form. This and other evidence has been construed to indicate that distinct protective mechanisms operate for larval and adult worms, necessitating separate analysis of each.

We had earlier shown that IgE was important in the expulsion of larval *T. spiralis* from the gut of rats. In her Ph.D. thesis research, Deborah Negrao-Correa showed further that specific IgE could effectively block the entry of the antigenically distinct adult *T. spiralis* into cells stimulated with interleukin (IL-) 4. This work has suggested entirely new processes by which IgE can function in the body against adult worms. Unfortunately, the isolation of IgE directly from the gut lumen proved technically difficult and yielded only very small amounts of usable IgE.

To continue this work, we decided to attempt to produce a protective monoclonal IgE with specificity for adult worms. We knew this would be a difficult undertaking

since very few monoclonal IgE's have ever been produced, despite considerable interest in this molecule.

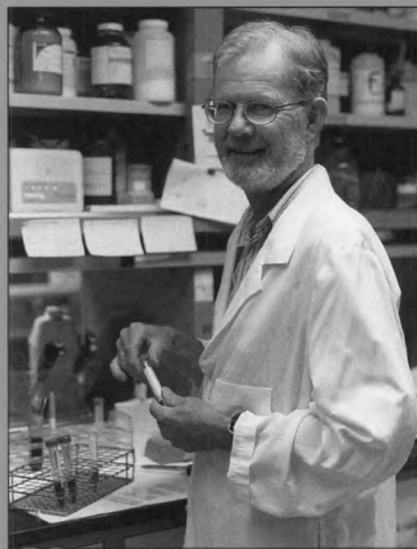
Monoclonal antibodies are produced by fusing a single antibody-producing B-lymphocyte with a tumor cell capable of indefinite growth. To date, we have conducted four fusions of immune B-cells and rat myeloma cells, producing more than 30 hybrids secreting IgE with anti-adult worm specificity. Two of these monoclonal antibodies appeared to be protective in our assay. However, the IgE-producing hybridomas were not able to sustain IgE production despite attempts to use IL-4 and other growth-enhancing cytokines that were known to be effective for IgE-secreting B-cells. This unusual behavior implies some previously unrecognized regulatory control on the continued synthesis and secretion of IgE antibody. Such

checks may exist to help keep IgE at its normal low levels in the blood.

During the course of these fusions we also screened for other hybrid cells producing antibody that could react with adult and larval *T. spiralis* antigens. We produced several IgG's, which await further analysis, and several IgA's. Unlike the IgE hybrids, the IgA hybrids prospered and we were able to produce substantial quantities of IgA for experimental use. We have tested this IgA for its activity in preventing larval worm penetration of the gut epithelium and found consistent, albeit not strong, levels of protection in rats. Since this IgA is in the dimeric molecular form, which is efficiently transported into the intestine, we believe that it is routinely processed and delivered to the lumen by the polymeric Ig receptor expressed on epithelial cells of the intestine and liver. These data thus add IgA to the growing list of immunoglobulins that can protect against nematode invasion. We now believe that one of the main functions of the T-cell activation process that follows worm infection is to provide the local intrainestinal conditions that facilitate the transfer of IgE, IgA, or IgG into the lumen. Once present, it appears that antibody of most, and perhaps any, isotype can be protective by preventing epithelial invasion by *T. spiralis*. Work on IgE has by no means finished and we are trying several new approaches to produce the monoclonal antibodies we need. However, the model system itself has proved extremely versatile in helping to unravel fundamental protective mechanisms that operate in the gut against nematode parasites. Our attention now will focus on precisely how these different antibody molecules achieve their effects on larval and adult worms.

—Robin G. Bell

*Recent evidence shows that *T. spiralis* changes its coating of antigens—the targets of antibodies—as it molts from the larval to the adult form.*



Robin Bell

Mucosal Immunity Laboratory

Nematodes are cylindrical worms with a primitive nervous system that they use to recognize their environment. Many nematodes are parasitic, including *Trichinella spiralis*, the species we have studied for many years in this laboratory. Our research has focused on the processes that the worm employs to invade its mammalian host, and the means by which antibodies can interfere with those processes, causing the parasite to be expelled from the host.

During the past year, postdoctoral associate Catherine McVay has shown that antibodies work in more than one way to expel worms. Dr. McVay inoculated larvae onto layers of cultured epithelial cells and then studied their invasion of those cells in the presence or absence of antibodies. She found that, in order to be protective, antibodies must bind to both the glycoprotein molecules on the surface of the worm and the related glycoproteins that are released from the mouths of the larvae. All of these molecules share a binding site for protective antibodies, and we think that one of the ways that the antibodies work is by cross-linking the molecules that are released from the mouth to the surface molecules.

Cross-linking of the parasite surface to the products released by the worm appears to be important to two mechanisms we observed in other experiments. Those studies showed that antibodies could completely block the worm from invading the cells. The antibodies appeared to accomplish this by covering the sensory receptors

around the mouths of the worms with large caps of antibody complexes. This seems to confuse the larvae, preventing them from recognizing the epithelial layer as a desirable location. Under slightly different circumstances, the antibodies encumbered the larvae that were already in the cell layer, preventing them from moving through the cells as they normally do.

Barbara Butcher, who joined our research group this year, has begun to study the ways in which parasite molecules interact with host cells. Her experiments show that some cells are resistant to physical invasion by the worm. However, resistant cells accept the glycoproteins released from the worms that are refused entry. The parasite molecules distribute within the cells in different ways. An unexpected finding was that some of the molecules enter the nucleus, something most pro-

teins do not readily do, while others are retained in the cytoplasm of the cell. We do not yet know what the glycoproteins do inside the cell. Dr. Butcher aims to compare the distribution of parasite molecules in resistant versus susceptible cells in order to gain a better understanding of the basis for susceptibility to invasion by the worm.

We have also investigated the signals that trigger the worm to invade epithelial cells. Jessica Geyer, a veterinary student from North Carolina State University, conducted these experiments when she joined our laboratory as a summer research fellow. Using electron microscopy, Lucy Gagliardo and I studied the precise location of the larvae when they invade cultured epithelial cells. Despite the very large body of the worm, which is one millimeter long and 30 microns wide in the mid-section, and the relatively small size of the cell—10 to 20 microns in diameter—the worm occupies the cytoplasm of the cell, or rather, a series of many cells. These accommodating cells appear to survive for only a short time following the departure of the worm, but we expect that the relationship between the worm and the cell is crucial to the development and growth of the parasite. The results of our investigations continue to impress upon us the devious and successful adaptations made by parasitic nematodes. Disrupting these interactions is in the best interest of the susceptible host and is the outcome of the successful immune responses that we study.

—Judith A. Appleton

*Our research focuses on the processes by which *T. spiralis* invades its mammalian host and how antibodies can interfere with those processes.*



Judy Appleton

Equine Genetics Center

Red for speed, strength, courage, and beauty for thousands of years, horses occupy a special niche among the domestic animals by virtue of their changing relationship with humans. No longer valued only for their utility, horses are now prized also as companions in sport and leisure. In the realm of science, bones of prehistoric horses make up one of the key sections in the fossil record of evolution. Thus, interest in the genetic history of the horse encompasses not only its domestication several thousand years ago, but extends back in time for millions of years.

HORSE GENOME PROJECT

Today there is intense interest in mapping the genes of many species, including the horse and its close relatives. Progress in equine gene mapping has been particularly rapid in recent years. In late 1995 an international collaborative effort of horse geneticists from 23 laboratories in 12 countries across the globe was begun. The Horse Genome Project was launched with a five-year commitment from the Dorothy Russell Havemeyer Foundation for support of yearly workshop meetings that are critical to the success of this cooperative undertaking.

During the past three years important information has become available about three different types of gene maps for the horse. A *physical* gene map contains details about the location of particular genes on individual horse chromosomes. Horses have 32 chromosomes, a relatively large number compared to many mammalian species. Only a handful of genes have been ascribed to each of these chro-

mosomes, and this is a very small fraction of the 50,000 genes estimated to make up the genome of a mammalian species. However, the genes already identified have helped to begin construction of a second type of map, a *comparative* gene map. This type of map is very useful when comparing well studied species, such as the human, with less well-studied species, like the horse. Data from the comparative map indicate that the linear arrangement of genes on horse and human chromosomes is very similar. This is very good news for those interested in horse genetics, because it means that much of the information from the human physical gene map can be applied to the horse, saving much effort for the horse geneticists.

The third type of gene map is the *linkage* map. Linkage maps contain informa-

tion on variability, or polymorphism, of genes. A linkage map consists of a collection of marker genes that are highly variable and also spaced rather evenly throughout the genome. Testing of families or individuals for these marker genes can be used to link these markers with traits of interest, such as disease susceptibility or performance ability. Linkage maps take advantage of the genetic variation in a species.

With the help of research support specialist Tatja Hopman, who joined our laboratory this year, we have been involved with the construction of a linkage map of the horse. Over the past two years we have identified 145 new markers, called microsatellites, which we are now characterizing for their polymorphism. Some of these genes have already been assigned places on the physical gene map, and they look promising as important markers of variability in horses.

Information from the physical, comparative, and linkage maps of the horse is being integrated to form a comprehensive gene map of this species. In the future this map should help horse breeders make informed breeding decisions that will improve the health and performance of their breeds.

REPRODUCTIVE IMMUNOLOGY OF THE MARE

How does a mother's immune system tolerate the presence of a growing fetus? Why, in most cases, does it not recognize and reject it, as it would a kidney or heart graft from the father of her developing child? The immunological paradox of "nature's transplant," as mammalian

Recent progress in equine gene mapping has been particularly rapid. Our laboratory has now identified 145 new markers.



Paige Adams

pregnancy has often been called, was first recognized in 1954 by Nobel Laureate Peter Medawar. Questions surrounding this paradox have occupied the minds of many scientists since that time, including those in our laboratory.

Many of the reproductive immunology studies of our laboratory over the past 20 years have centered around the chorionic girdle, a fascinating component of the horse placenta discovered in 1897 by E. Cossar Ewart of Edinburgh University. In 1997 the members of my laboratory had the opportunity to participate in a scientific conference convened in Edinburgh by the Havemeyer Foundation to celebrate the centennial of Professor Ewart's discovery.

Postdoctoral associate Sarah Temaner described her studies of gene regulation in the chorionic girdle. Dr. Temaner's work is leading to a better understanding of how the placenta controls the expression of fetal antigens that can induce graft rejection. By suppressing the expression of these antigens, the developing horse embryo can avoid detection, and therefore rejection, by the immune system of the mother.

Maria Viveiros, another postdoctoral associate in our laboratory, discussed her studies of cytokines, the small messenger molecules made and secreted by cells for signaling and communication. Placental cells, including those of the chorionic girdle, secrete cytokines that can influence the type of immune responses an individual can make. Dr. Viveiros's work has shown that chorionic girdle cells produce at least two important cytokines of the immune system. Our

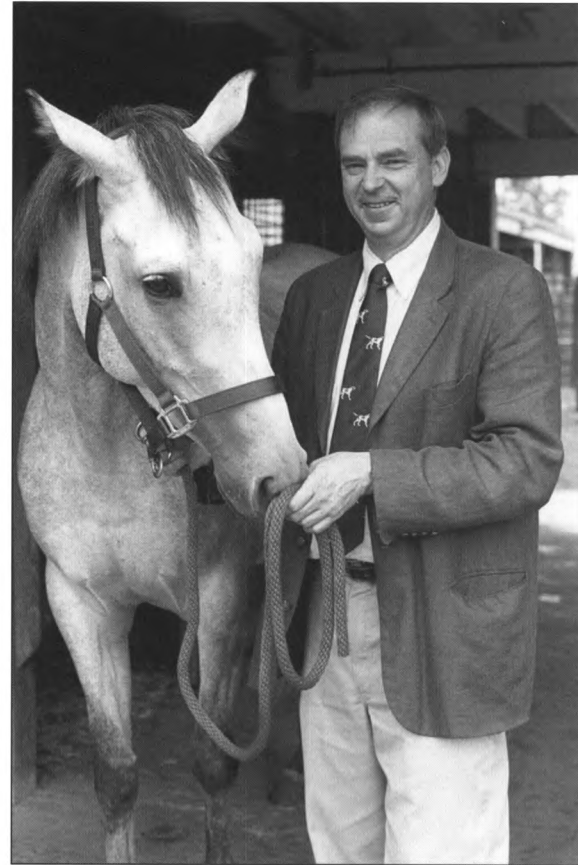
work to determine the role of these cytokines in the maintenance of equine pregnancy is continuing.

Jessica Baker, a second-year graduate student, presented her studies of maternal immune reactivity during pregnancy. Ms. Baker has discovered that the character of immune responses made by pregnant mares differs from those made when they are not pregnant. She has tested cultured white blood cells, or leukocytes, for their capacity to recognize and destroy leukocytes from a different individual. Such assays mimic certain aspects of transplantation reactions. Ms. Baker has found that the ability of the mare's leukocytes to destroy foreign cells from the mating stallion is decreased in pregnancy. Further research will be needed to determine whether cytokines produced by the

We have found that the ability of the mare's leukocytes to destroy foreign cells from the mating stallion is decreased in pregnancy.



Maria Viveiros



Doug Antczak and Bold Jane

conceptus cause these changes in leukocyte reactivity.

Mammalian pregnancy involves the reproductive system of the mother, the rapidly developing fetus, and the placenta that surrounds the fetus in the uterus. Knowledge of reproductive and developmental biology, immunology, and genetics are required in the study of this most complex, and most essential, aspect of life. The research team of the Equine Genetics Center is well poised to make important advances in this fascinating area of biology and veterinary medicine.

— Douglas F. Antczak



A Retrospective

IT IS A STRANGE FEELING to sit down to write my final report after 33 memorable years at what began as the Veterinary Virus Research Institute. What an exciting time it was! It began before Vietnam, before “flower power” and Birkenstocks. Those 33 years culminated last September at the retirement “symposium” that Doug Antczak and his outstanding staff organized for Skip Carmichael and me. I shall never forget it! I also wish to extend my sincere gratitude to my wife, Barbara, and my children, to Mary Beth Matychak, my trusted helper for 24 years, to all my graduate students, ending with Reinhard Straubinger, who recently took over my lab, to my colleagues, and to Skip Carmichael and the late James Baker, who took me aboard in 1964 and whose support and collaboration over the years were remarkable. Since then I have experienced the growth of the Baker Institute both in buildings and, more importantly, in its staff and internationally renowned faculty with its ever expanding research endeavors. It is the outstanding environment and camaraderie of the Baker Institute that made the difference and created a fertile ground in which to do good work.

For me, it all started in the spring of 1964 when Skip Carmichael came to Brockville, Ontario for a veterinary conference. I was an employee at the Animal Disease Research Institute in Ottawa, where I had gone to begin a research career after spending four years in large and small animal practice in Germany. I quickly discovered that I needed more than a veterinary degree for research. Andy Greig, a virologist at the ADRI, put me in touch with his former graduate advisor, Dr. Baker. Andy had first

Opposite: Max Appel in the 1980s.

worked under Dr. Baker during World War II, when then-Captain Baker headed up a classified rinderpest research project for the U.S. and Canadian governments on Grosse Isle, an island in the St. Lawrence River. Dr. Baker sent Skip Carmichael to Canada to look me over, and, thanks in part to the good Canadian beer, I became Skip's first graduate student that fall.

The project and the financing for my Ph.D. program came from a typical James Baker arrangement. Dr. Baker had met a wealthy Fifth Avenue physician, Oswald Jones, who had a theory that persistent virus in patients who had become infected with measles as adults increased their risk of developing pulmonary emphysema. Since canine distemper virus (CDV) is closely related to measles virus, Dr. Baker suggested a study in dogs to confirm the observation. Dr. Jones provided the funds and I went to work. The hypothesis did not materialize, but it began my career of research with CDV, which brought ever new surprises over the years.

I received my Ph.D. degree in virology in the spring of 1967 and accepted Dr. Baker's offer to stay at Cornell as an assistant professor. It was an endowed professorship, again in the typical fashion of Dr. Baker, who raised the funds for each faculty position at the Institute. My colleagues at that time, Skip Carmichael and Ben Sheffy, had similar positions. Jim Gillespie, who earlier held a post at the Institute, had just left to create the Feline Health Center in the Microbiology Department of the College of Veterinary Medicine.

The early years of the Institute were an exciting time for virology. Dr. Baker created specific-pathogen-free colonies of cattle, swine, and dogs for the study of infectious

diseases, and by the 1960s it seemed as though a new virus was isolated every month if not weekly. Very little was known about canine viruses, but this changed fast. Dr. Carmichael had just isolated a canine herpesvirus that caused up to 100 percent neonatal mortality in affected litters. Dr. Binn at the Walter Reed Army Institute reported on the isolation of canine adenovirus, canine parainfluenza virus, and the bacterium *Bordetella bronchiseptica* from dogs with kennel cough. Dr. Ditchfield in Canada had isolated a canine adenovirus from dogs with tracheobronchitis that differed from the canine adenovirus that causes infectious canine hepatitis. I developed a method for isolating virulent CDV from dogs with acute distemper.

Although tissue culture for the isolation of viruses had been introduced earlier in

the century by Alexis Carrell, bacterial contamination greatly hampered its use until antibiotics became available in the 1940s. Dr. Baker brought the technique to Cornell from the Rockefeller Institute in 1947 and established a central tissue culture laboratory that attracted visitors from around the world. Initially, all virus isolation, titration, and neutralization were done in small glass tubes or flasks that had to be washed for re-use. One person was responsible for the weekly preparation of hundreds of tubes. It took another full-time technician just to perform canine distemper virus neutralization tests in embryonating hen eggs. In the 1970s, 96-well microplates were introduced, greatly simplifying the tissue-culture process. Each well in a microplate took the place of one egg in the former test. Many changes and improvements in techniques were yet to come.

My graduate students performed much of the laboratory work that contributed to our progress. The first was Marilyn Menegus, who later became director of the diagnostic laboratory at the University of Rochester Medical Center. She worked with canine adenovirus type 2 (CAV-2), the virus that Drs. Ditchfield and Binn had isolated from dogs with kennel cough. It turned out that CAV-2 causes a predominantly local infection in the lungs, in contrast to CAV-1, which causes a generalized infection and fatal hepatitis. However, CAV-2 proved to protect dogs against infection with both viruses. This observation resulted in a change in the composition of dog vaccines from CAV-1 to CAV-2, a project that we completed together with Skip Carmichael. Skip had demonstrated that "blue eye" and persistent kidney infections were undesirable responses to the old CAV-1 vaccine. They were eliminated by the introduction of CAV-2 vaccines.

The outstanding environment and camaraderie of the Baker Institute created a fertile ground in which to do good work.



Max Appel and Mary Beth Matychak in 1977

In the years to follow we concentrated our efforts on kennel cough, which was widespread at that time and virtually uncontrolled. David Bemis, now a professor of microbiology at the University of Tennessee, was involved in the study. It turned out that *Bordetella bronchiseptica* was the main culprit, enhanced by viral infections of canine parainfluenza virus and sometimes CAV-2. The *B. bronchiseptica* organisms attach to the cilia in the trachea and the bronchial tree, a remote site for systemic immune responses and blood-borne antibiotics to reach. Because mucosal immunity is essential for protection against these agents, the intranasal vaccine developed during the next decade proved to be superior to parenteral vaccines. I sometimes wonder why intranasal whooping cough vaccines for children are not developed. Whooping cough is caused by *B. pertussis* and the pathogenesis is similar.

When I came to the Institute, immunology as a separate discipline did not exist. Little was known about the function of lymphocytes other than that they made antibody. That changed dramatically in the 1970s. The advances since then have been mind-boggling.

When Bill Shek came aboard as a graduate student in 1974, little was known about cellular immune responses in viral infections. He developed a method to test the effect of cytotoxic T-cells on CDV-infected cells, an important aspect in the pathogenesis of the disease. Graduate student Shaw Tsai established that the presence of interferon in cerebrospinal fluid was an indicator for persistent CDV infection of the brain. Joe Friedlander, my only graduate student who is now in private veterinary practice, made the interesting observation



ALLEN RADFORD

that CDV remained virulent when it was passaged in B-cell lines but could be largely attenuated by passage in T-cell lines.

For the past 20 years I have been collaborating with Brian Summers, and now, looking back, I don't know what I would have done without him. He came from Australia as my graduate student in 1976 and later became a professor of veterinary pathology at Cornell. Brian joined our lab at the time when Adalbert Koestner and his group in Columbus, Ohio made the observation that different "biotypes" of CDV existed, one that causes acute grey-matter brain disease and another, more subacute, that causes a white-matter disease with demyelination similar to that seen in multiple sclerosis (MS). With our own field isolates we could confirm their observation and Brian established the model for MS, which brought us funding from NIH and from the MS Society. After Brian completed his Ph.D., we continued to collaborate on projects, such as studies comparing the behavior of CDV strains in cultured brain cells. This work was largely performed by Sue Pearce-Kelling, who

A springtime "ticking" expedition in Westchester County, New York, 1991. Clockwise from left: Max Appel, Woo Lee, Martin Wiedmann, Beata Mizak, and Allen Radford.

worked in the lab for many years and recently joined Gus Aguirre's group.

When Brian joined the lab in 1976, Dr. Baker had passed away and Douglas McGregor was taking over the directorship of the newly named James A. Baker Institute for Animal Health. Dr. Baker had always provided us with funding for our research: "You give me results and I give you the money." We were spoiled. All of that changed under the new directorship, and, as a Christmas gift, I found an NIH grant application form in my mail box!

The next problem with canine distemper virus appeared in wildlife. By the 1970s, black-footed ferrets were almost extinct, in part because of exposure to CDV. When a colony was found in the Dakotas, the ferrets were trapped and vaccinated with the modified-live distemper vaccine intended



An all-star line-up. From left: George Lust, Gus Aguirre, Max Appel, and Skip Carmichael.

for dogs. It killed them all. Another colony was found in the 1980s in Wyoming, but natural infection with CDV had wiped out all but six animals. These were trapped and kept in isolation. Because an inactivated CDV vaccine was not available on the market, we produced one to protect the ferrets. Thanks to breeding efforts, there are now more than one thousand black-footed ferrets distributed in several zoos and returned to the wild, all of them derived from the original six animals.

Vaccination with modified-live CDV caused a similar problem in zoos, where carnivores like the red panda and several other species also succumbed. For the last 20 years Mary Beth Matychak has produced protective vaccine for these animals in our laboratory at the Institute.

In 1988 Ab Osterhaus in Rotterdam and others investigated the outbreak of a virus disease that killed 90 percent of the harbor seals in the North Sea. The virus

turned out to be closely related but not identical to CDV.

In the 1990s some javelina (*Tayassu tajacu*) in Arizona showed signs of a central nervous system disorder that we diagnosed as CDV. Javelina, which are also known as collared peccaries, look like pigs but are unrelated to them. Interestingly, they are also susceptible to rinderpest virus, a morbillivirus closely related to CDV that causes severe disease in cattle.

A few years later we investigated a disease outbreak in lions, tigers, and leopards in the Wildlife Waystation in San Fernando, California, and again found CDV. For hundreds of years canids with distemper were in contact with felids without spreading the disease—why now? We still don't have the answer. The virus in the big cats proved to be identical to a virus isolated from nearby raccoons.

Two years later, a disease outbreak killed one-third of the lions in the Serengeti Reserve in Tanzania. Samples submitted by Steve O'Brien from the NIH and Linda

Munson, then at the University of Tennessee, contained CDV. Brian Summers saw it first in stained tissues. We then isolated the virus from lions as well as hyenas and domestic dogs from the area and found classical CDV in all. Ab Osterhaus, who had received all my earlier CDV isolates, reported on small differences between isolates from North American carnivores including large cats and those from Europe and Africa.

In the 1980s the emphasis in virology shifted from the study of viral proteins and their functions to the study of viral nucleic acids. Jeff Mitchell, who is now an assistant professor at the University of Missouri College of Veterinary Medicine, was the first to apply the techniques of molecular biology to our distemper model after he came to my lab as a graduate student in 1983. He produced DNA and RNA probes from the virus for *in situ* hybridization, an important new step for CDV work at the time. Now, in the 1990s, genetics has taken over with its potential for gene therapies. What does the next decade have in store?

The most exciting time in my career came in the fall of 1978, when we isolated a novel canine parvovirus that caused a fatal hemorrhagic enteritis in dogs, a new worldwide disease epidemic. Hundreds of people called the Baker Institute with reports of sick or dying dogs and asking for our help. The parvovirus epidemic resulted from a mutation in a parvovirus of another species, probably feline panleukopenia, that allowed it to enter the canine population.

Helen Greisen, our electron microscopist, spent countless hours on the instrument searching for the small virus particles. She was a botanist by training, having earned masters and doctoral degrees at Cornell. After Helen retired in 1983 she worked as a volunteer at the

Cornell Plantations, where an annual memorial lecture was established after her death last year.

A hectic two years followed the discovery of the parvovirus, culminating in the development of vaccines in collaboration with Skip Carmichael. The resulting patents were quite beneficial to Cornell, the Institute, and us. Paul Meunier, a graduate student in my laboratory at that time, studied and published on the pathogenesis of the disease. Institute professor Colin Parrish, who was a graduate student studying under Dr. Carmichael during that time, is now recognized as a world expert on the molecular make-up of canine, feline, and other parvoviruses.

In 1991 Dr. McGregor initiated the admirable Leadership Training Program for veterinary students. In a ten-week period during the summer, the program offers students with potential for a career in research an opportunity to do "hands on" laboratory work. One of my summer students, Reinhard Straubinger from Munich, later returned to the States with a Mercedes Foundation grant (but not a car) after receiving his veterinary degree in Germany. Earlier this year he completed his Ph.D. in my laboratory and has been directing research there since I retired in September.

When Reinhard came to my laboratory, we had recently established a canine model for Lyme disease, a chronic infection of humans and dogs caused by a spirochete, *Borrelia burgdorferi*, that is transmitted by ticks. The initial studies, including protection by vaccination, were made in collaboration with our colleagues in the Diagnostic Laboratory: Rich Jacobson, Sang Shin, and Yung Fu Chang; entomologists Sandy Allan and Lisa Patrican; and, as always, Brian Summers from the Pathology Department. Other summer students, including Martin

Wiedmann and Thomas Vahlenkamp, as well as a visiting veterinarian from Poland, Beata Mizak, were involved in the early Lyme research. They all will remember the sunny days in the woods in Westchester County where we collected *B. burgdorferi*-infected ticks for our studies. Reinhard, in recent years joined by his veterinarian wife, Alix Straubinger, and a postdoctoral fellow from Germany, Luc Härter, concentrated his studies on the pathogenesis of Lyme arthritis and antibiotic treatment. His early results garnered the laboratory a five-year contract from the NIH for Lyme disease research.

Besides research, I always enjoyed teaching and the stimulating contact with students. In the earlier years Skip Carmichael and I taught a course for graduate students in virology, which Colin Parrish later took over. In 1989, when former Dean

Poppensiek retired, I took over his course on foreign animal diseases. My experience with these diseases came from the years at the Animal Disease Research Institute in Canada, where I was involved in the preparation of demonstration courses for veterinarians on foreign animal diseases at Grosse Isle, the Canadian equivalent of Plum Island.

Grosse Isle has a fascinating history. It was the quarantine station for European immigrants during the last century, where countless people died from infectious diseases. Later I took a course on Plum Island itself. When the curriculum change in the veterinary college was introduced several years ago, that course was added to the curriculum. With the new curriculum I became a tutor for Block IV, an enjoyable task I hope to continue.

My time at the Institute has been interspersed with much travel around the world. It has been a tremendous learning experience over the years to meet people and to see so many countries. Every profession has an advantage and I always considered this to be the icing on the cake in mine.

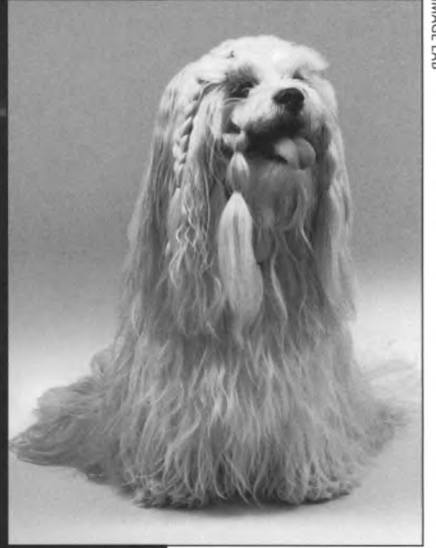
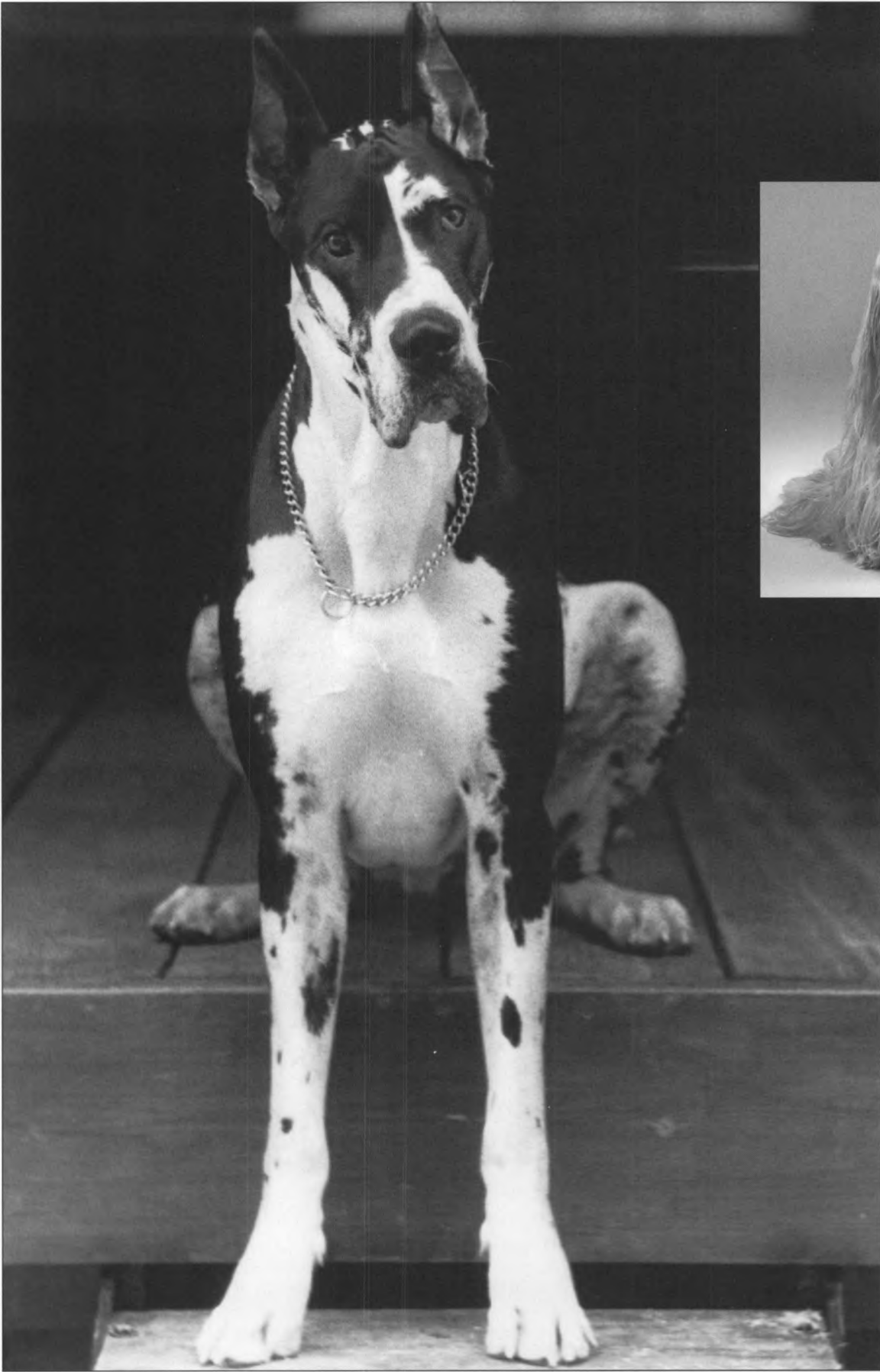
It is time now to step aside. It is my hope that my replacement will have a genuine interest in infectious diseases, especially those of the dog, which were the main focus of Skip Carmichael's and my own work. If not, who else will tackle the next epidemic? After all these years of learning I still identify with Professor Borg, a character in the Ingmar Bergman film "Wild Strawberries," when his teenage niece concludes, "Uncle, you are an emeritus professor but you don't know anything!" I will continue to work on that.

—Max J. G. Appel

The most exciting time in my career came in 1978, when we isolated a novel canine parvovirus.



RUSS HAMILTON / CU

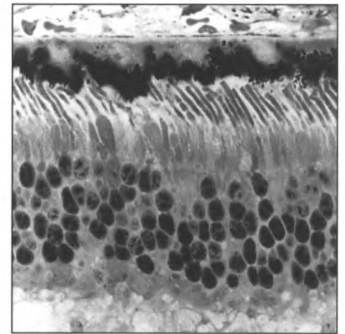


Genetics and Development

THE EVOLUTIONARY PROCESS has filled the earth with a multitude of distinct species, each with unique characteristics. The striking diversity of nature is a source of continual wonder and delight to those who study it, whether as amateurs or professionals. But within this natural diversity, most individuals of any wild species seem remarkably similar in size, form, and coloration.

Contrast the uniformity of a flock of geese with the enormous variety of sizes, shapes, and coat colors among selectively bred domestic animals such as sheep, cattle, and horses. In the space of a few thousand years—or only a few hundred—selective breeding has wrought great changes in form and behavior. We have tapped the genetic diversity within the domestic species and shaped it with results very similar to the evolutionary process—breeds of dogs, cats, cattle, and horses breed as true as species in the wild.

The domestic species thus represent a wonderful experiment in selection, with much to teach us about genetic variation.



Opposite: Nowhere is the variation in one species more remarkable than among dogs.

Right: Normal rod and cone cells in the canine retina. Many dog breeds are susceptible to PRA, a group of inherited diseases that cause these cells to degenerate or fail to develop normally.

Human experimentation has taught us an unfortunate lesson as well—that genetic defects are often carried along when selection for particular traits is intense. In the reports in this section we describe the Institute’s efforts to understand the molecular basis of variation and to identify ways to prevent the defects that lie hidden within the genetic codes that determine the make-up of our livestock species and our animal companions.

John M. Olin Laboratory for the Study of Canine Bone and Joint Diseases

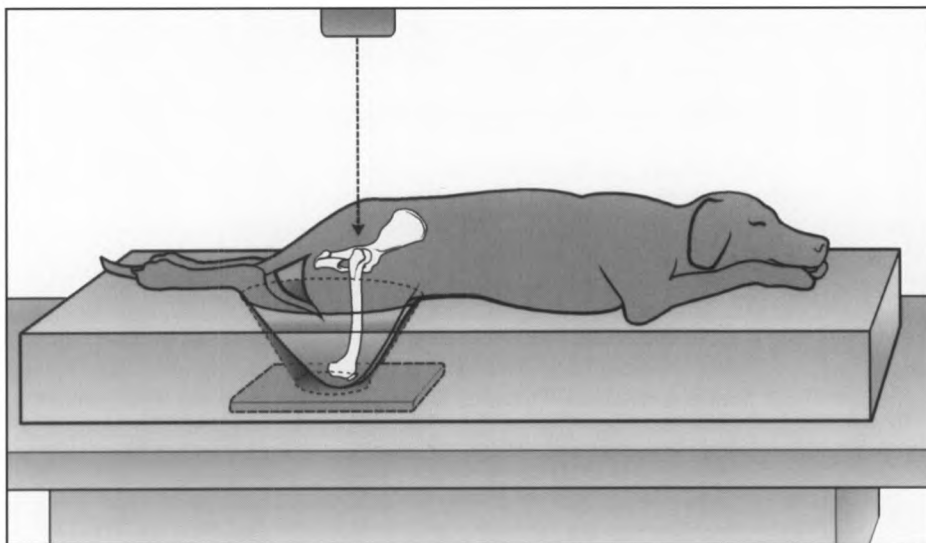
A collaboration between this laboratory and two members of the Department of Clinical Sciences has yielded promising new insights about the diagnosis and pathogenesis of hip dysplasia and osteoarthritis in dogs. Technician Alma Williams and I have been working for two years with surgical resident James Farese and assistant professor Rory Todhunter to devise a radiographic method to assess the degree of functional instability present in the hip joint of a dog that is standing or walking. The procedure we have devised, the dorsolateral sub-

luxation (DLS) test, attempts to determine the extent of functional joint instability by measuring the degree of subluxation, or displacement, of the femoral heads that can be observed when a dog is in a kneeling position.

Dogs are anesthetized or sedated before being examined. They are then placed with their chests in a foam rubber mold and their hindlimbs in a kneeling position. The stifles are flexed back so that the lower legs rest on the mold, with the femurs perpendicular to and in contact with the table. The hips are then X-rayed in this weight-bearing position, as shown in the figure.

The amount of displacement for each hip is scored as the percentage of the femoral head that is covered by the joint socket, or acetabulum, in this position. In a group of Labrador retrievers we tested, the scores ranged from 68 percent down to 30

percent. The higher percentage indicates coverage of a greater portion of the femoral head by the acetabulum and therefore greater joint stability. When we examined dogs at eight months of age, dysplasia-free and dysplastic hip joints



In the DLS test position, a dog rests in a foam rubber mold on the X-ray table. The dotted-line arrow indicates the path of the X-ray beam.

clearly fell into two groups. The ten hip joints we classified as disease-free had a mean score of 64 percent, plus or minus three percent, while the eight joints classified as dysplastic had a mean score of 39 percent, plus or minus three percent.

Our preliminary data suggest that the DLS test evaluates internal hip joint conformation as a quality that is distinct from joint laxity as judged by the PennHip® method. To further evaluate the clinical utility of the DLS test, we are examining the relationship between a DLS score given at eight months of age and the eventual status of a dog's hips after maturity.

Dr. Todhunter and Ms. Williams made another interesting observation in studying Labrador retrievers. They noted an association between changes in developmental patterns in the cartilaginous

growth region near the femoral heads of newborn pups and the later onset of hip dysplasia. In puppies that were destined to develop normal hip joints, the ossification center that forms bone from cartilage in the femoral heads appeared by day 12 after

birth. In puppies that were genetically at risk for hip dysplasia, the ossification centers did not appear until 15 to 25 days after birth. We are currently testing the data for a relationship between the delayed onset of femoral head ossification and a low DLS score at eight months of age.

If these data are confirmed, the date of appearance of a femoral head ossification center might provide a new and strikingly early means to predict either normal or dysplastic hip joint development in dogs.

—George Lust

Our goal is to understand the biochemical and mechanical changes that occur in cartilage very early in the development of osteoarthritis. As dog owners and breeders know well, osteoarthritis is the unavoidable consequence of hip dysplasia. In time it destroys the cartilage and involves other soft and bony tissues of the joint.

The repeated application of heavy load stress to normal cartilage, or of normal load stress to unhealthy cartilage, can damage the matrix surrounding the cartilage cells and is thought to be important to the early development of osteoarthritis.

Postdoctoral fellow Chih-tung (Chris) Chen, a bioengineer who has joined our laboratory, is analyzing the early biochemical responses of cultured cartilage samples to matrix damage. Dr. Chen has found that the rate of loading, or compression, is a critical determinant of damage. The impact force that jackhammer operators and boxers, for example, might experience is more destructive than a smoothly arising compression of the same magnitude. Dr. Chen has shown that the collagen fibers, important constituents that contribute to the mechanical strength of the cartilage, are injured at high loading rates. The evidence for this includes an increase in water content as well as the staining of histological sections of the cartilage by a special antibody that detects denatured collagen.

Dr. Chen and John Bertram of the Department of Anatomy are continuing to develop an even more sensitive method to detect subtle and early changes in matrix structure. This method involves a novel testing device, termed the vibratory probe, that measures mechanical properties of cartilage at very high frequencies (20–300 hertz, or cycles per second). Preliminary evidence indicates that measuring mechanical properties over a range of these high frequencies produces spectra that are characteristic of the condition of the cartilage. In other words, we could potentially have a “fingerprint” for cartilage in the early stages of degeneration that cannot be obtained at the lower frequencies to which traditional mechanical testing equipment is limited. If the principles on which this vibratory probe are based can be validated in ongoing studies, there is potential for adaptation to instrumentation for use in minimally invasive arthroscopic procedures.

The radiographic methods described by Dr. Lust in this report, and especially the computerized tomography illustrated in last year’s annual report, allow us to visualize changes that occur over time in joint geometry. One of these changes involves pressing of the rim of the acetabulum into the cartilage adjacent to the fovea, the site on the ball of the hip joint where cartilage lesions are known to initiate. Thus we are able to study cartilage in a presumptive early lesion, prior to the appearance of damage but after the joint has begun to experience abnormal loading patterns.

It is not unreasonable to suppose that the cartilage in these joints has begun to experience the kinds of destructive loading that Dr. Chen is studying in culture. Our findings are very interesting. Water content and swelling suggest that the cartilage at the site of lesion predilection is

already weaker in all dogs regardless of risk status. The computed tomography imaging supports initiation of lesions by unaccustomed mechanical factors at this inherently weaker site. Among the earliest biochemical abnormalities at the lesion site are an elevated fibronectin content and a focal loss of proteoglycan near the articular surface.

The protein fibronectin in cartilage has long been an interest of our laboratory, since levels of this protein can increase over 20-fold in severely fibrillated osteoarthritic cartilage. With our colleagues in Jamie MacLeod’s laboratory we recently identified a new isoform of this protein in articular cartilage. Technician Caroline Borden developed a precise means to quantitate expression of this isoform in eight different cartilage tissues of the body including the ear, trachea, disk, rib, and nose as well as in the meniscus and articular cartilage of joints. Finding this isoform in all cartilages looked at, but not in any non-cartilage tissues, confirmed that the (V+C)- fibronectin, as we have named it, is a cartilage-specific protein. Furthermore, Ms. Borden showed that expression of this isoform is lost rapidly when cartilage cells are removed from their cartilaginous matrix, declining from 54 percent to less than five percent after the cells have been cultured for only three days without an intact matrix. These findings support an important role for this fibronectin isoform in cartilage biology and could have implications not only for understanding the pathogenesis of cartilage lesions but also for promoting their healing.

—Nancy Burton-Wurster

Our identification of a new cartilage-specific fibronectin isoform could have implications for understanding and healing cartilage lesions.



Nancy Burton-Wurster and George Lust

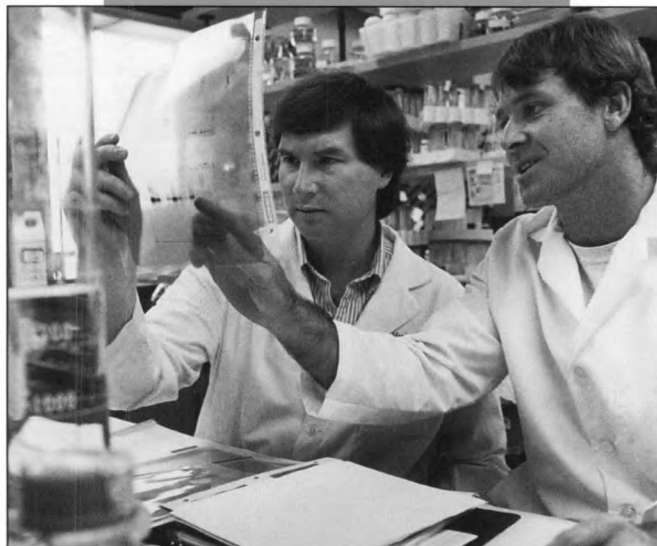
Laboratory of Cellular Growth and Differentiation

There are several different cartilaginous tissues in the body. Rib cartilage, articular cartilage on joint surfaces, and structural cartilage in the ear and nose are all relatively stable throughout life. In contrast, other tissues exist only temporarily in the form of cartilage before changing into bone by a process called endochondral ossification. In fact, most of the bones in our bodies form initially as cartilaginous structures. After birth, these same bones continue to grow in length at specific sites called growth plates, which are also made of cartilage. Even healing fracture sites pass through a cartilaginous stage before mineralizing into new bone.

In biomedical research, a very common experimental strategy is simply to investigate similarities and differences between two related items. There are many examples: normal function compared to a disease process, young compared to old, one species compared to another. Not surprisingly, important clues to new knowledge are often found by studying not only what is different, but also what is the same. We rely on this general comparative strategy for much of our work on cartilage. While all cells that synthesize cartilage are called chondrocytes, there are basic differences between the chondrocytes in stable articular cartilage and those in the transitional growth plate cartilage destined to form bone.

Matthew Stewart, a veterinarian with specialty training in surgery, has been comparing chondrocytes from different cartilaginous tissues since joining our laboratory in 1994 to pursue a Ph.D. degree. The focus of Dr. Stewart's research has been regulation of chondrocyte proliferation. This is a very important basic question relevant to problems in cartilage regeneration, bone growth both before and after birth, and bone fracture healing. He has demonstrated differential expression of critical cell-cycle regulatory genes, the molecular switches that determine whether or not a cell will divide. The data demonstrate that chondrocytes need to arrest cell proliferation in order to fully express a differentiated phenotype. In addition, different types of chondrocytes respond in unique ways to certain systemic and local regulatory signals.

The regulation of chondrocyte proliferation is relevant to problems in cartilage regeneration, bone growth, and fracture healing.



Jamie MacLeod and Matt Stewart

This work on cartilage cell biology complements our other ongoing projects on the biochemical properties and expression patterns of cartilage matrix proteins. Looking to the future, efforts in this laboratory will concentrate on trying to understand the normal dynamic interaction between chondrocytes and the cartilage matrix, how diseases such as osteoarthritis interrupt these relationships, and how therapeutic intervention can be optimized to restore normal function.

We have success to report in another area of our research. The first clinical trial studying safety and efficacy of our recombinant canine erythropoietin preparation has been completed. The data clearly demonstrate the ability of this canine hormone to stimulate red blood cell production in dogs, while avoiding the serious and sometimes life-threatening complication of immunogenicity seen frequently when dogs are treated with human erythropoietin. These results promise new hope for the treatment of nonregenerative anemia in dogs secondary to kidney failure, cancer, and other chronic diseases, and several pharmaceutical corporations

have expressed an interest in this work. We are hopeful that recombinant canine erythropoietin will eventually become commercially available to all veterinary practitioners.

—James N. MacLeod

Laboratory for the Study of Inherited Canine Reproductive Diseases

Our laboratory has been interested for several years in a disorder of dogs known as XX sex reversal (XXSR). This reproductive abnormality is inherited as a recessive trait. Carriers are clinically normal and can be male or female. Affected dogs have a normal female chromosome constitution but develop ovotestes or testes. The rest of the reproductive tract then also develops abnormally, and infertility is a common result. Despite the development of testicular tissue, however, it is often difficult to differentiate sex-reversed dogs from normal females, even during a spay operation, so the cause of the infertility can go undetected. The long-term goal of our research is to develop a DNA test that will allow us to clearly identify both carrier and affected dogs, providing a practical method of improving breeding performance in susceptible breeds.

OUTREACH TO BREEDERS AND VETERINARIANS

This year we conducted a mail survey of breeders and veterinarians in the United States. Our initial purpose was to increase breeders' awareness of XX sex reversal by describing the common findings in affected dogs. The survey has already resulted in publication of several related articles in newsletters for breeders and for veterinarians. We are using the information we have collected to estimate the prevalence of XXSR and its impact on breeding performance in various breeds. We are providing confidential diagnostic assistance to breeders, owners, and their veterinarians. As a result of the survey we have been able to

identify affected dogs in breeds in which XXSR was not previously reported. These new breeds are being included in the search to find the gene that causes this disorder.

SABBATICAL LEAVE: MOLECULAR STUDIES IN ENGLAND

For the first nine months of 1997 I was continuing a year-long sabbatical leave in Cambridge and London. While in Cambridge I took a course for genetics researchers, "Linkage Analysis and Molecular Biology Computing," that was given at the Human Genome Mapping Program Resource Center. Linkage analysis is a method used to identify an unknown gene by comparing DNA of individuals in a family. This method was used in humans to find the genes causing Huntington's disease and cystic fibrosis. We are using this technique, along with others, to find the gene

that causes XX sex reversal in dogs. In Dr. Peter Goodfellow's laboratory in Cambridge, we began linkage analysis by screening American cocker spaniels in a pedigree containing XXSR-affected members. We also began cloning the canine counterparts of genes that are known to be involved in testis and ovarian differentiation in humans and in mice. Both the linkage analysis and the canine gene cloning are continuing in our laboratory at the Baker Institute.

I spent the second half of my sabbatical leave studying with Dr. Robin Lovell-Badge, head of the Laboratory of Developmental Genetics at the National Institute for Medical Research in London. This laboratory is renowned for discovering genes that control the embryonic development of the reproductive system. Members of this laboratory and their collaborators discovered the mouse *Sry* and *Dax* genes that are critical for differentiation of the testis and ovary, respectively. There I learned the specialized molecular techniques needed to analyze embryonic gene expression.

I was made to feel welcome by everyone I met in England, and it was a wonderful scientific and cultural experience. I was fortunate to be in London in August, the only month when Buckingham Palace is opened for tours. The palace is a virtual museum and it was thrilling to see the original portraits of queens and kings that I had only seen in history books. This was a truly memorable way to end my sabbatical in England.

— Vicki N. Meyers-Wallen

We are collecting information about the prevalence of XX sex reversal (XXSR) and its impact on breeding performance in various breeds.



Vicki Meyers-Wallen

Donnelley Laboratory of Gene Regulation and Expression

Research in this laboratory seeks to determine the biochemical and molecular bases of several inherited disorders found in dogs, cats, and primates. Our goals in studying these diseases are to develop molecular diagnostic techniques to distinguish normal, carrier, and affected individuals and to develop strategies for gene therapy. The diseases we pursue are mucopolysaccharidosis, a potentially fatal disorder caused by a lysosomal enzyme deficiency; macular degeneration, which affects central vision, ultimately causing blindness; and oculo-skeletal dysplasia, which impairs vision and causes skeletal deformity.

Our report this year focuses on oculo-skeletal dysplasia in dogs, a problem we began studying last year. Oculo-skeletal dysplasia is known to occur in several breeds of dogs, most notably Labrador retrievers and Samoyeds. In both of these breeds, affected dogs have skeletal abnormalities, characterized as short-limbed dwarfism, together with ocular defects, including vitreous dysplasia, retinal detachment, and cataracts. The ocular abnormalities cause blindness in most affected dogs. This combination of defects is inherited in both breeds as an autosomal recessive disorder, meaning that affected individuals inherit two copies of the defective gene. Interestingly, carriers

of this disease, those with one copy of the defective gene and one normal gene, still exhibit a congenital retinal defect that can be seen with an ophthalmoscope. Vision in these individuals may be normal or impaired.

Although the expression of the disease follows the same type of inheritance pattern in both Labradors and Samoyeds, Institute colleague Gregory Acland found that cross-breeding an affected Samoyed with an affected Labrador did not produce dwarf offspring. This breeding study suggests different causes for the disorder in the two breeds. With the information provided by the pedigrees that Dr. Acland has been developing, we are now looking for the gene defect or defects that cause this disorder in these two breeds.

Canine oculo-skeletal dysplasia resembles Stickler syndrome type 1 in humans.

Using information from new pedigrees, we are looking for the gene defect or defects that cause oculo-skeletal dysplasia in Labradors and Samoyeds.

About 65 percent of reported human Stickler syndrome cases are related to a defect in the gene that codes for type II collagen, a major component of both the vitreous body of the eye and of cartilage. Similarities in pathology between Stickler syndrome and the oculo-skeletal dysplasia in Samoyeds and Labrador retrievers have suggested the possibility that the mutation or mutations responsible for the canine disease might be found in the type II collagen gene.

In order to test this theory, however, it was first necessary to clone and characterize canine type II collagen cDNA. Fuliang Du, a graduate student in our laboratory, has finished the cloning work and characterized a genetic marker in the gene. Using this marker we have ruled out the possibility that the type II collagen gene is responsible for the disease of Samoyeds, but it is still a candidate gene for oculo-skeletal dysplasia in the Labrador retriever.

We are continuing our search for a molecular defect in the type II collagen gene in our Labrador retriever pedigree and searching for other possible candidate genes that might be responsible for

the dwarfism present in Samoyeds. Success in identifying the molecular defect should shed light on the events taking place in the cells that cause both the visual and skeletal abnormalities to develop.

— Jharna Ray



Jharna Ray and Fuliang Du

Inherited Eye Disease Studies Unit

The past year has been tremendously rewarding for our laboratory in several areas of our studies of canine progressive retinal atrophy (PRA). We are making significant progress in looking for markers linked to rod-cone dysplasia 2, a disease of collies. We have found a sequence variant in affected miniature schnauzers that may in part explain the inheritance of photoreceptor dysplasia. Most importantly in terms of the number of dogs that might benefit, our progress to identify the molecular basis of progressive rod-cone degeneration (*prcd*) has been truly remarkable.

The most widespread inherited retinal blindness of dogs, *prcd* develops after maturity in animals that are born with normal vision. The difficulty of identifying carrier or affected dogs before they produce offspring has led to a significant rise in the incidence of the disease.

Two years ago our laboratory undertook a collaborative effort with Elaine Ostrander's group at Fred Hutchinson Cancer Research Center to make a framework reference map of the dog using microsatellites generated in her laboratory. For mapping, we used many three-generation families, which included 163 F² offspring. Over half the dogs came from retinal disease pedigrees, primarily *prcd*, in which affected-to-carrier matings had been performed in very outcrossed and polymorphic individuals.

As a result of this collaboration we have found numerous microsatellite markers and four different genes that are linked to *prcd* in our test pedi-

grees as well as in pedigrees we have tested from outside our colony. We have ruled out a causal role with *prcd* for three of the genes, but the fourth is a potential candidate, and work to test and sequence this gene is currently in progress.

By itself, a microsatellite-based linkage map of the dog is not sufficient to clone a disease gene. To circumvent this limitation, we have assisted Dr. Ostrander's group in establishing a panel of canine-rodent cell hybrids to help determine gene order and synteny between the human, mouse, and dog maps for conserved regions of the genome. As a product of these studies, we have localized the *prcd* gene locus to canine chromosome 9, which is homologous to most of the q arm of human chromosome 17. Although fine mapping of this region in dogs has not been completed, the conservation of gene

order in this interval between dog and man is striking. Our working hypothesis is that *prcd* is the canine counterpart of a form of human retinitis pigmentosa, RP17, although it may also turn out to be a novel retinal disease caused by a defect in a nearby locus.

The discovery of so many markers and genes that are linked to *prcd*, and whose order in a small segment of the dog genome is known, will permit us to develop a linkage-based DNA test to determine whether any dog is PRA affected, a carrier, or genetically normal. Because we have identified so many markers that flank the *prcd* locus, we anticipate that the linkage test we are developing will be completely accurate and can be carried out on individual dogs rather than pedigrees, as linkage testing typically requires.

Our long-term objectives are to identify the gene responsible for *prcd* and to characterize the defect at the molecular level. Achievement of these objectives will be essential to understanding how the mutant gene causes disease and to developing gene therapy methods and, in the shorter term, an unequivocal, mutation-based DNA test.

Based on the progress made recently, we feel confident that we will have available to owners and breeders a DNA-based linkage test for the *prcd* form of PRA within the next year, possibly sooner. With reasonable progress and success, we anticipate that the *prcd* gene and defect will be identified soon after, and a mutation-based test can be developed and made available.

—Gustavo D. Aguirre

Our progress in identifying the molecular basis of progressive rod-cone degeneration (prcd) has been truly remarkable.



Weiquan Wang and Weikuan Gu



Beyond the Laboratory

SCIENTIFIC RESEARCH may involve many long hours of solitary experimentation in the laboratory, but it is by no means a solitary pursuit. For knowledge to advance, it must be shared. The faculty and students of the Baker Institute engage in frequent scientific interaction with their colleagues in the broader scientific community.

They do this first by teaching and by publishing their findings in peer-reviewed journals. They also travel throughout the country and abroad to lecture and confer with their colleagues in other universities, public health laboratories, and industry. A variety of seminar series at the College of Veterinary Medicine and elsewhere within the University ensure that a steady stream of experts from other institutions are brought to Cornell to share their findings with their colleagues here. The number and quality of these exchanges give a true measure of the value of a research program or institution.

In the following pages we summarize some of the more noteworthy activities of our scientific staff and some of the recognition they have received as a result of their research efforts.

Opposite: Just visiting... Long-time collaborators Theo Van Veen (left) and Gus Aguirre. Professor Van Veen, a retinal disease expert, is here on sabbatical leave from Gothenburg University in Sweden.

Baker Institute Scientific Conference Series

Leading scientists from major research centers across the United States and from Australia to Slovenia gathered at Cornell in July 1997 for the second annual Baker Institute scientific conference, "Canine Genetics: The Map, The Genes, The Diseases." The meeting provided a major opportunity for the international canine genetics community to pool their knowledge about the rapidly developing framework map of the dog genome and the powerful molecular techniques that are making it possible for geneticists to integrate the physical and linkage maps that several groups are constructing.

Three groups, the European DOGMAP consortium, Jasper Rine's laboratory at the University of California, Berkeley, and Elaine Ostrander's group at the Fred Hutchinson Cancer Research Center in Seattle, announced the development of framework, or low resolution, linkage maps of the dog genome. Dr. Ostrander's group has collaborated for several years in this effort with members of Gus Aguirre's laboratory at the Baker Institute. Five months after the meeting, the Ostrander and Aguirre groups published the first framework reference map of the canine genome. The availability of their markers and those that others have mapped will aid in the identification of linked traits,

whether desirable or harmful, and allow researchers to focus on the identification of the genes, and the mutations within those genes, that are associated with disease in dogs and humans.

progress towards the cloning of these and more retinal diseases of dogs.

The workshop was organized by Institute faculty members Gus Aguirre, Greg Acland, and Kunal Ray with help from pro-



Members of the Institute's Inherited Eye Disease Studies Unit

At the meeting, members of Dr. Aguirre's laboratory announced that the developing canine genetic map had been used to show very tight linkage of markers and genes to specific regions of the canine genome for two forms of progressive retinal atrophy, early retinal degeneration (*erd*) in the Norwegian elkhound and progressive rod-cone degeneration (*prcd*) in multiple breeds including the miniature poodle, Labrador retriever, English and American cocker spaniels, and Portuguese water dog. No gene mutation has yet been identified in either disease, but the physical mapping resources developed and reported at this meeting will facilitate

gram committee members Elaine Ostrander, Jasper Rine, Matthew Binns of the Animal Health Trust in Newmarket, England, and Urs Giger of the University of Pennsylvania. The workshop was co-sponsored by Cornell's College of Veterinary Medicine, the Eugene V. and Clare E. Thaw Charitable Trust, the American Kennel Club Canine Health Foundation, Amersham Life Sciences, Inc., Dad's Products Company, Inc., ICOS Corporation, Kal Kan Foods, Inc., the Orthopedic Foundation for Animals, PE Zoogen, the Wellcome Trust, and the Foundation Fighting Blindness.

Honorable Mentions

FACULTY HONORS

Gustavo Aguirre was honored with an "Excellence in Canine Research Award" at the annual meeting of the American College of Veterinary Internal Medicine in May, 1997. The award was presented by the American Veterinary Medical Association Council on Research and sponsored by the American Kennel Club. As part of the awards program, Dr. Aguirre gave an invited lecture, "Inherited Eye Diseases in Dogs and the Coming of Age of Molecular Ophthalmology."

Institute director and professor **Douglas Antczak** and Francesca Stewart of the Babraham Institute, Cambridge, England co-organized a scientific meeting convened by the Dorothy Russell Havemeyer Foundation in Edinburgh, Scotland. The conference on trophoblast differentiation marked the centenary of the discovery of the equine chorionic girdle by Prof. E. Cossar Ewart.

Robin Bell was elected in 1997 to a three-year term on the Cornell University Faculty Senate.

The canine program of the International Veterinary Vaccines and Diagnostics Conference held in July at the University of Wisconsin, Madison was dedicated to virology professors **Leland Carmichael** and **Max Appel**. This recognition of their distinguished contributions to the field of canine infectious disease research came in anticipation of their retirement later in the year. The conference organizer, University of Wisconsin professor Ronald D. Schultz, was a member of the Baker Institute faculty from 1973 to 1977.

In September Dr. Carmichael received the Outstanding Service Award of the New York

State Veterinary Medical Society "in recognition of his outstanding contributions to the advancement and improvement of veterinary medicine in New York State."

Judith Appleton was the recipient of a prestigious Chancellor's Award for



A self-portrait by Mindy Story

Excellence in Teaching from the State University of New York. Dr. Appleton was the only awardee in 1997 from any of Cornell's four statutory colleges. The award recognized her contributions to the teaching program in microbiology and immunology in the College of Veterinary Medicine. Dr. Appleton also served as the University's director of graduate studies in immunology from 1994 through 1997.

Colin Parrish was invited to present an overview of his studies of canine parvovirus host range at a special meeting on Interspecies Transplants at the National Institutes of Health in Bethesda, Maryland. Laboratory members **Wendy Weichert** and **Dai Wang** presented the results of their studies at the International Parvovirus workshop in Heidelberg, Germany, and **John Parker** and **Wen Yuan** presented their work at the American Society for Virology Meeting in Bozeman, Montana.

STUDENT HONORS

Jessica Baker was awarded a National Needs Graduate Fellowship, an institutional training grant in animal biotechnology sponsored by the United States Department of Agriculture. Ms. Baker is studying the effects of pregnancy on the maternal immune system in the laboratory of Douglas Antczak.

Also in Dr. Antczak's laboratory, Havemeyer Foundation Summer Fellow **Mindy Story**, a veterinary student from Colorado State University, won first prize in the Molecular Biology section of the Veterinary College's Summer Leadership Training Program competition for her summary presentation of her research.

DEGREES CONFERRED

Reinhard Straubinger, Ph.D.: "The Pathogenesis of Acute Lyme Arthritis in Dogs." Dr. Straubinger is continuing this work as a research associate at the Baker Institute.

Deborah Negrao-Correa, Ph.D.: "The Intestinal Immunoglobulin E Response to Infection with *Trichinella spiralis* in Rats." Dr. Negrao-Correa is now an assistant professor of immunology at the Institute of Biological Sciences of the Federal University of Minas Gerais in Brazil.

Michael Olivier, Ph.D.: "Molecular Genetic Studies on Canine Hip Dysplasia." After doing post-doctoral research at the Institute, Dr. Olivier will continue his work with complex inherited diseases as a post-doctoral associate in the laboratory of Dr. David R. Cox, Department of Genetics, Stanford University School of Medicine. Dr. Olivier will study the inheritance of hypertension in humans.

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Several of our benefactors are due special mention. We received exceptional support this year from Cully Ray, a first-time contributor, the Mrs. Cheever Porter Foundation, which made a second generous equipment grant this year, and from our “old” friends Jacquie Lindsay, David Behnke and Paul Doherty, the Albert C. Bostwick Foundation, Judy and Fred Wilpon, and Sue and Neil Van Sloun. We are enormously grateful to all of them.

Sports Illustrated might seem an unlikely sponsor of an institute for animal health, but our friends at the magazine made it possible for the Institute to host sky box receptions this year at the Westminster Kennel Club Dog Show and the National Horse Show in New York City. Both events were held at Madison Square Garden, where *Sports Illustrated* just happens to have the best view of center court. We are especially grateful to Bob Kohansky of *Sports Illustrated* and Hank Travis, the chairman of our Advisory Council, for making this opportunity a reality.

Opposite: Jim Hardy with a new arrival at the Institute's McConville Barn.

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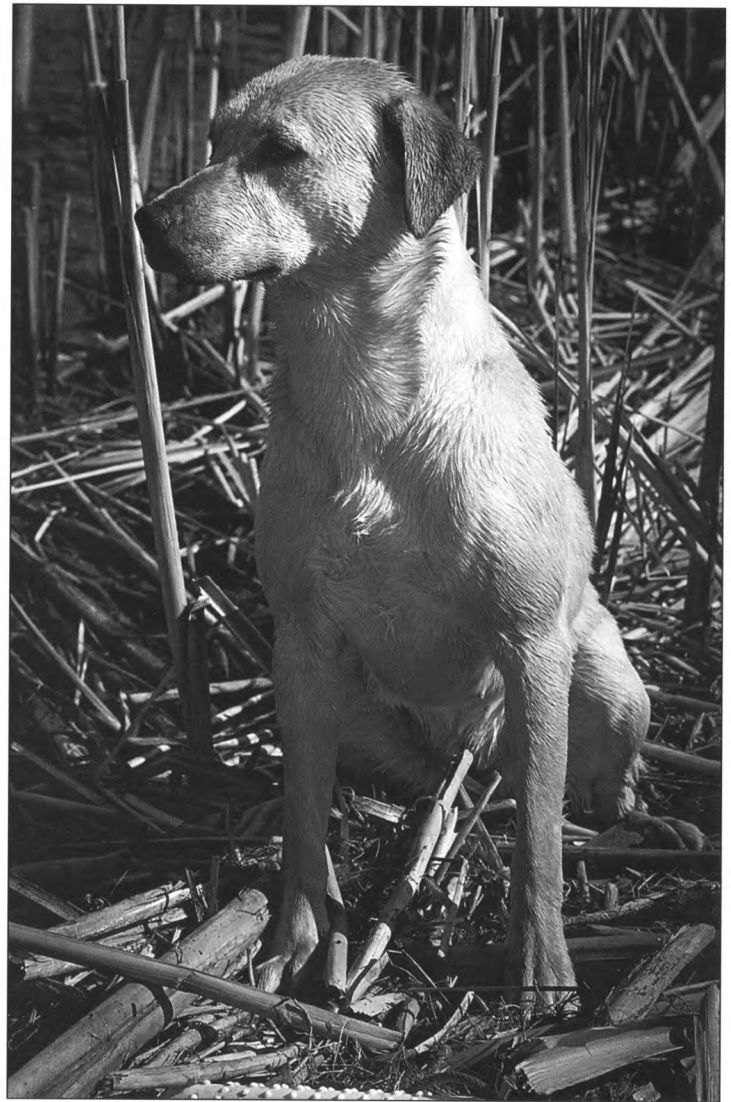
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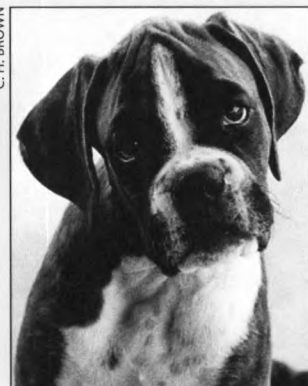
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