James A. Baker Institute for Animal Health Cornell University Annual Report, 1988

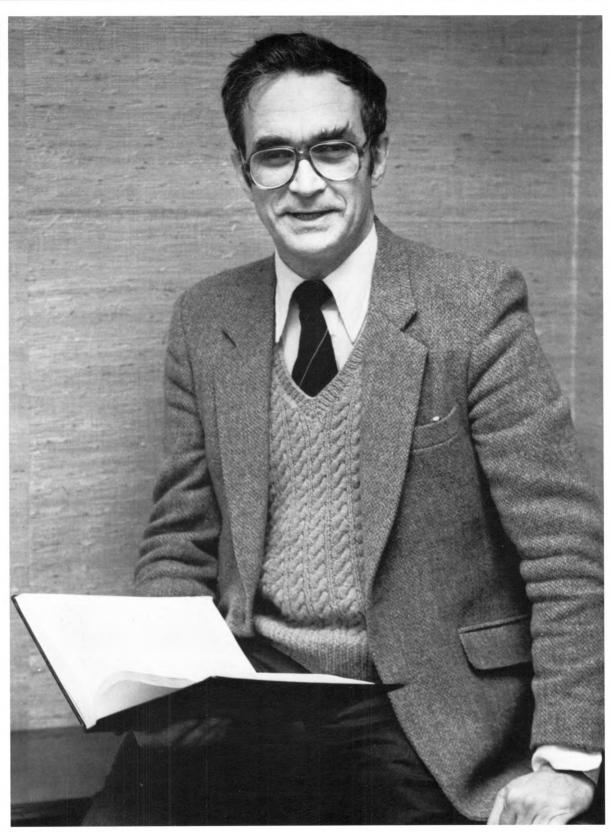
New York State College of Veterinary Medicine Volume 38



This report honors those whose generosity sustains the Institute's independence and commitment to excellence and the pursuit of truth.



For nearly forty years, the Baker Institute has been an innovator and a significant participant in animal health research.



Douglas McGregor

Research has been a decisive factor in shaping our society. Nowhere is that more clearly evident than in the biomedical sciences. For nearly forty years, the Baker Institute has been an innovator and a significant participant in animal health research. Immediately after the Institute's founding in 1950, novel methods of cell and tissue culture were used to isolate infectious agents, grow these agents in quantity, and modify them for use in vaccines. Baker Institute scientists still employ these methods to advantage; however, they are also using contemporary methods of molecular biology to address fundamental questions about life processes. The principal areas of their research are in three areas in which the Institute has a historic and ongoing commitment: infectious diseases, arthritis, and reproductive biology. Their most recent achievements are described in this report.

The technology on which modern science depends has many applications in animal health research, but it is also expensive. Last year I commented on the significant investments we have made in facilities and equipment. In September we completed a major capital improvement program that addressed the Baker Institute's immediate space and instrument needs. The centerpiece was a new research wing that contains two laboratory suites and badly needed space for offices and shared equipment. Coincident with construction of the new wing, three laboratories were refurbished and supplied with instruments provided by the National Science Foundation, the SURDNA Foundation, the Robert W. Woodruff Foundation, and several private benefactors.

While facilities and equipment are required to conduct research, they alone are not sufficient. People with skill, energy, and imagination are needed to use these resources in practical and creative ways. It is therefore with great pride and gratitude that I can report to you that the Baker In-

stitute received two gifts this year that will directly assist our faculty. A Career Development Award established by Mr. Elliot Donnelley, Mr. Strachan Donnelley, and Ms. Laura Donnelley in honor of their mother, Mrs. Dorothy R. Donnelley, will provide assistance for and continuing support of new faculty and established scientists who are making major shifts in the direction of their research. As chairwoman of our advisory council, Mrs. Donnelley has lent guidance and encouragement that have contributed to the unprecedented growth of the Institute during the past decade. In addition, the trustees of the Marilyn M. Simpson Charitable Trusts made a gift in honor of Mrs. Simpson, whose concern and affection for animals have been recognized in earlier awards to the Institute. We are deeply grateful to the individuals who made these gifts, and we are confident that their investment will be richly rewarded in accomplishments that will benefit the health of dogs and horses.



Anita Hesser

The Baker Institute has a staff of talented men and women, a dedicated advisory council, and a growing number of concerned benefactors.

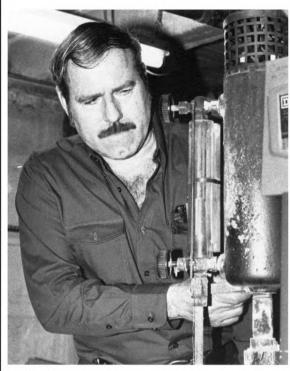


Susan Hamlin

At the annual meeting of the advisory council, Mr. Atherton Bristol was named recipient of the 1988 Arthur F. North, Jr. Canine Service Award. The award recognized Mr. Bristol's generosity and foresight in establishing the Bristol Fund for Canine Research. Created in memory of his Dandie Dinmont Terrier, Queechy, the fund will provide ongoing assistance for research conducted in our Cornell Research Laboratory for Diseases of Dogs. Other individuals and clubs have made similar investments in the future. I wish to express special thanks to those individuals and kennel clubs who contributed to the Muriel and Herbert Kaye Memorial Fund for Canine Research for their leadership in establishing named endowments. Their gifts are testimony to the kind of bond created by a shared concern for dogs and form a lasting tribute to the Kayes.

As we reflect on the significant events of 1988, a basic lesson to consider is that the Baker Institute can be no better than those who staff it or those who promote its research and training missions. The Institute is well endowed in that respect. It has a staff of talented men and women, a dedicated advisory council, and a growing number of concerned benefactors. An institution could wish for no greater asset.

Douglas D. McGregor



Arthur Howser



Carlene Furch

Staff of the Baker Institute

Administration

Douglas D. McGregor, director: B.A., M.D., U. of Western Ontario; D. Phil., Oxford U.

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Lorelei J. Dyess, administrative aide: B.S., West Texas State U.

Anita S. Hesser, secretary to the director Florence C. Huth, administrative aide

Sharon E. Morrow, accounts assistant

Jeanne G. Truelsen, assistant director for development: B.A., M.A., Miami U.

Robert D. Wakeman, office assistant

Emeritus

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Laboratories

Giralda Laboratory for Canine Infectious Diseases

Leland E. Carmichael, John M. Olin Professor of Virology: A.B., D.V.M., U. of California; Ph.D., Cornell

Maria Fernandez-Maillo, graduate research assistant: D.V.M., U. Complutense, Madrid Catherine Y. Linder, graduate research assistant: Baccalaureate, European School (Brussels);

D.V.M., Veterinary School, Toulouse

Jean C. Joubert, research support specialist

Hadley C. Stephenson Laboratory for the Study of Canine Diseases

Max J. G. Appel, professor of virology: Dr. med. vet., U. of Hannover; Ph.D., Cornell

Mary Beth Matychak, research technician: U. of Evansville

Susan E. Pearce-Kelling, laboratory technician: B.S., M.S., Cornell

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

George Lust, professor of physiological chemistry: B.S., U. of Massachusetts; Ph.D., Cornell

Nancy Burton-Wurster, senior research associate: B.A., M.S., Ph.D., New York U.

Harry R. Leipold, graduate research assistant: B.S., Cornell

Rory J. Todhunter, graduate research assistant: B.V. Sc., Sydney U.; M.S., Michigan State, Dip. ACVS, Cornell

Juergen Steinmeyer, postdoctoral fellow: Ph.D., U. of Bonn

Elizabeth Grisanzio, laboratory technician: B.S., U. of Vermont

Margaret S. Peery, laboratory technician: B.S., Ohio State U.

Raymond O. Recchia, laboratory technician: B.S., State U. of New York, Fredonia

Alma J. Williams, laboratory technician: B.A., U. of Pennsylvania; M.S., Cornell; AALAS accreditation

Immunology Laboratory

Robin G. Bell, associate professor of immunology: B.Sc., Australian National U.; Ph.D., John Curtin School of Medical Research

Ching-hua Wang, research associate: M.D., Peking Medical School; Ph.D., Cornell

Ali Ahmad, graduate research assistant: D.V.M., M.Sc., U. of Agriculture (Faisal Abad, Pakistan)

Karen M. Trotter, postdoctoral associate: B.S., M.S., Ph.D., Cornell

Duzhang Zhu, graduate research assistant: M.D., Anhui Medical College

Lincoln S. Adams, research technician: B.S., Hobart College; AALAS accreditation

Mucosal Immunity Laboratory

Judith A. Appleton, assistant professor of immunology: B.S., Indiana U.; M.S., Ph.D., U. of Georgia

Melissa S. Carlisle, graduate research assistant: B.V.Sc., U. of Queensland Lucille F. Gagliardo, laboratory technician: B.S., Southhampton College Lynn A. Usack, laboratory technician: B.S., Cornell

Equine Genetics Center

Douglas F. Antczak, associate professor of immunology: B.A., Cornell; V.M.D., U. of Pennsylvania; Ph.D., Cambridge U.

William L. Donaldson, graduate research assistant: B.V.Sc., U. of Queensland Julio G. Oriol, graduate research assistant: D.V.M., U. Nacional de Buenos Aires Chong-Hui Zhang, graduate research assistant: M.S., Nanjing Agricultural U. Debra A. Dougherty, research support specialist: B.S., U. of Rhode Island Christine L. Pelkaus, laboratory technician: B.S., Cornell

Donnelley Laboratory of Immunochemistry

Douglas D. McGregor, professor of immunology: B.A., M.D., D. Phil. **Alan D. King,** graduate research assistant: B.S., U. of Alabama; D.V.M., Auburn U. **Mark D. Hulsebosch,** laboratory technician: B.S., St. Joseph U.

Bostwick Laboratory of Molecular Biology

Colin R. Parrish, assistant professor of virology: B.Sc.; Massey U.; Ph.D., Cornell

Research Service Laboratories

Thomas M. Bartol, Jr., research support specialist (flow cytometry): B.S., Cornell **Paul J. Lutwak**, research support specialist (chromatography): B.A., B.S., Miami U.

Glassware

Jeannette R. Carney, laboratory attendant **Barbara J. Kokke**, laboratory attendant

Animal Care

Roy L. Barriere, vivarium supervisor: AALAS accreditation

Jack H. Arcangeli, Jr., animal technician

William Campos, animal technician: AALAS accreditation Raymond M. Combs, animal technician: AALAS accreditation

Raymond J. Corey, animal technician: A.A.S., State U. of New York Agricultural and Technical College, Delhi; AALAS accreditation

Kevin T. Draiss, animal technician: B.S., Cornell; AALAS accreditation

Louise E. Eller, animal technician

James C. Hardy, animal technician: B.S., Cornell; AALAS accreditation

Laura M. Michel, animal technician: A.A.S., State U. of New York Agricultural and Technical College, Farmingdale; B.S., Cornell; AALAS accreditation

Anastasia E. Newell, animal technician: AALAS accreditation

Maintenance

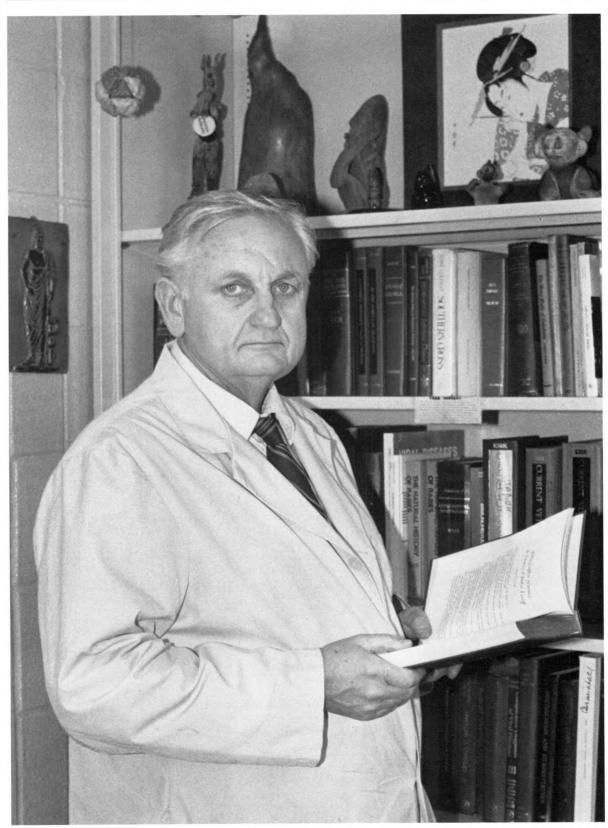
Arthur D. Howser, maintenance supervisor, buildings and grounds

Richard E. Daniels, maintenance mechanic

Robert A. Foux, maintenance mechanic

Russell F. Haus, maintenance mechanic

We are attempting to define the role of minute virus of canines (MVC) as a cause of enteric illness in young pups and its effects on the pregnant bitch.



Leland Carmichael

Giralda Laboratory for Canine Infectious Diseases

One aspect of our research entails the characterization of a little-known parvovirus of dogs, the minute virus of canines (MVC). This year we reported that MVC is distinct from canine parvovirus type 2 (CPV-2), the highly pathogenic virus of dogs that was first identified ten years ago. Dr. Maria Fernandez-Maillo, a Ph.D. candidate in this laboratory, has developed improved methods for detecting the virus. She also has prepared a library of monoclonal antibodies that will allow further characterization of MVC and its relationship to other parvoviruses.

Major objectives of our research are to determine whether MVC (also known as CPV-1) can cause reproductive disease or enteric illness in dogs. Approximately 80 percent of tested animals have been found to have antibodies to MVC, yet it has only recently been linked to enteric illness and death of pups less than two weeks old. The association was unexpected because experimental infections were mild. Since other parvoviruses can cause fetal infections, we are now focussing our attention on MVC infection of pregnant bitches and its effects on their newborn pups.

Canine Parvovirus Type 2: The Well-known Killer

As part of our continuing investigation of CPV-2, we examined several ill-founded views regarding CPV-2 infection and immunization strategies that have been advocated in commercial advertisements and dog fancy magazines. Our findings led to the following conclusions:

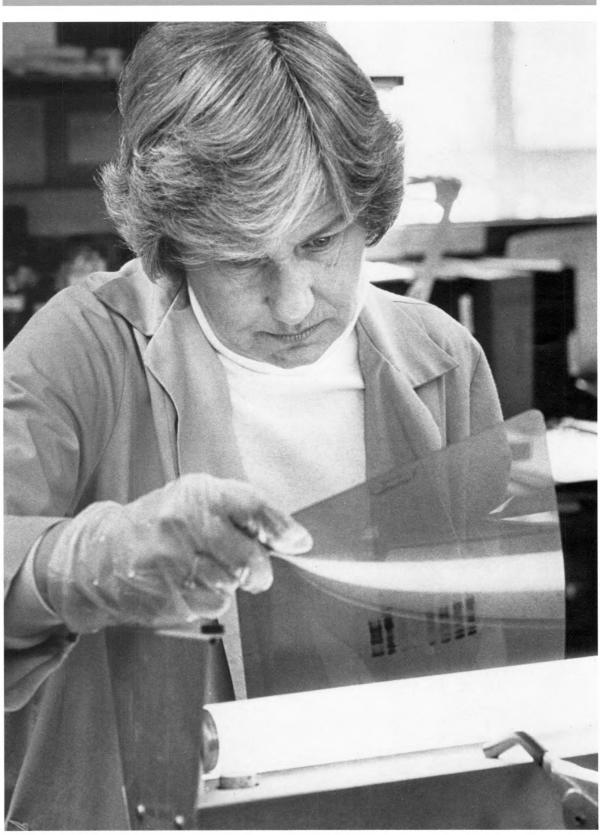
 Attenuated live vaccines vary in their capacity to immunize. They differ with regard to the minimal immunizing dose and to the rate and level of protective antibodies they induce in vaccinated dogs. Most live vaccines immunize satisfactorily. None of those tested caused clinical illness.

- Neither virulent nor vaccinal strains of CPV suppress or interfere with the immune response to other components of multiple vaccines such as distemper, hepatitis, or parainfluenza virus.
- None of several attenuated CPV-2 vaccines that we tested immunized dogs at a time when they were susceptible to infection with virulent viruses. However, individual vaccines did differ in their capacity to immunize pups with different levels of maternal antibodies. To reduce the prevalence of infection in breeding kennels, it is necessary to both isolate and vaccinate pups.
- Although Dobermans and Rottweilers appear to be especially susceptible to severe parvoviral disease, their antibody responses to CPV-2 vaccines are not significantly different from those of other breeds that receive the same vaccine.



Florence Huth

We examined several ill-founded views regarding canine parvovirus type 2 (CPV-2) infection and immunization strategies.



Jean Joubert

- "Booster" responses did not occur with any vaccine in dogs that had hemagglutination-inhibiting antibody titers greater than 1:80. Dogs with lower antibody titers had variable responses after revaccination. Four- to eightfold differences in antibody titers were reported on samples of the same serum submitted to different laboratories.
- "New" vaccines are not needed to protect dogs against CPV-2. All currently licensed vaccines contain viruses similar to strains isolated in 1978. Living vaccines, chemically inactivated vaccines, and nonliving recombinant vaccines prepared from 1978-type viruses all protect against virulent CPV-2 strains,

including recent variants that are more virulent than those isolated several years ago.

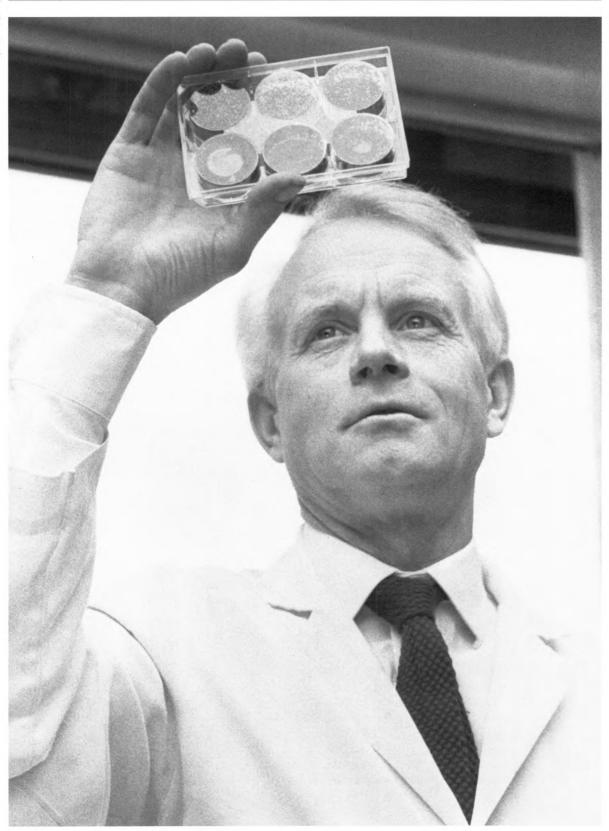
Canine Herpesvirus

The major immunogenic proteins of canine herpesvirus (CHV) were partially characterized by Catherine Linder, who completed her M.S. degree in the fall. By knowing which proteins are significant in inducing immunity, it will be possible to analyze CHV strains for use as vaccines. Another potential benefit would be to develop monoclonal antibodies that can protect newborn pups against CHV in kennels where fatal infection is common.

Leland E. Carmichael



We have tested a panel of monoclonal antibodies specific for canine distemper virus (CDV).



Max Appel

Hadley C. Stephenson Laboratory for the Study of Canine Diseases

Distemper

In 1988, a distemper epidemic killed approximately 1,000 Huskies in the Canadian and Greenland Arctic and about 80 percent of the harbor seals in the North Sea. To better study such epizootics, reagents are needed to distinguish different strains of virulent distemper virus. Monoclonal antibodies, exquisitely specific antibodies that can detect very slight differences among molecules, may be the tools we seek. Therefore, we have tested a panel of monoclonal antibodies specific for canine distemper virus (CDV). We have not vet found antibodies with the distinguishing power we need; however, we are now testing other monoclonal antibodies obtained from Dr. Erling Norrby of the Karolinska Institute of Stockholm.

The antibodies provided by Dr. Norrby have a high capacity to neutralize CDV. We have found that dogs treated during the incubation period up to eight days after infection with virulent CDV were fully protected from the disease; antibody treatment had no value thereafter. We concluded that such monoclonal antibody treatment might be useful in protecting unvaccinated dogs that have been exposed to CDV but would not benefit animals that already developed clinical signs of illness.

In related work, we have been studying the effect of distemper infection on myelin, a lipid-rich material that insulates nerves in the brain and spinal cord much the way that insulating materials coat the wire of an electrical cord. Distemper in dogs and multiple sclerosis in humans are associated with a loss of myelin. In collaboration with Dr. Brian Summers, we have, using the electron microscope, examined the very early changes in CDVinfected dogs. We observed that in myelin-rich areas the virus first infects glial cells, causing the myelin sheaths to swell and disorganize. Macrophages, the immune system's scavenger cells, then move in to remove the damaged myelin.

Lymphocytes are latecomers to the scene. They appear to attack virus-infected glial cells rather than the myelin sheaths. These studies suggest that the loss of myelin is a consequence of CDV infection producing glial-cell injury rather than an autoallergic response, as has been suggested in multiple sclerosis.

In the meantime, a morbillivirus that appears to be related to CDV has been isolated from cattle. Although it is not known whether the virus causes disease, a seroepidemiological survey is underway to determine its distribution in healthy dairy and beef cattle in the United States.

Synthetic Materials for Surgical Implants

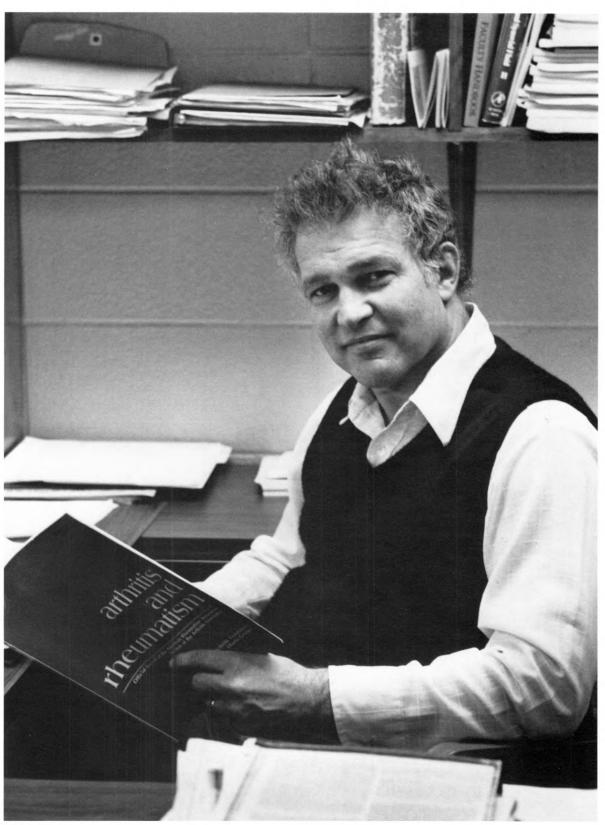
Typically, synthetic materials used in vascular surgery, hernia repair, or heartvalve replacement, for example, are rejected by the body as foreign substances. Once the materials are enveloped by the body's own fibroblasts and endothelial cells, however, they are no longer recognized as foreign. Macrophages, the first cells attracted to such "grafts," are responsible for sending out signals that provoke fibroblasts to attach and multiply. In collaboration with Drs. Chu and Jeong Lim, we have been testing the attachment and activation of macrophages and fibroblasts on thirteen synthetic fabrics. We have noted significant differences among them that could prove important in choosing materials for the fabrication of surgical implants.

New Book

Virus Infections of Carnivores (to be volume one in the series Virus Infections of Vertebrates) was completed this year. We contributed chapters and edited the book, which covers virus infections of dogs, cats, mink, ferrets, nondomestic carnivores, and pinnipeds.

Max J. G. Appel

A new position for pelvic X rays of dogs looks promising in helping to predict whether pups will later develop hip dysplasia.



George Lust

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

Hip Dysplasia

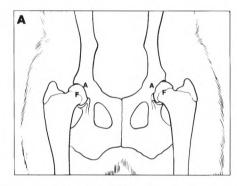
A new position for pelvic X rays of dogs looks promising in helping to predict whether pups as young as four months old will later develop hip dysplasia.

Although a standard pelvic X-ray examination is useful with adult dogs, it is less accurate in diagnosing dysplasia in growing dogs. We have had encouraging results with a new position for hip-joint X rays developed by Drs. Gail Smith and Darryl Biery of the University of Pennsylvania Veterinary College in Philadelphia. Instead of the standard legs-extended position, anesthetized dogs are placed with their hind limbs perpendicular to the tabletop. While an object is put between the thighs, inward pressure is exerted at the knees. This manipulation forcibly displaces the femoral head (ball) from the acetabulum (socket) of the joint. By measuring the distance that the femoral head is displaced from its normal position, the hip can be assessed as either normal or pre-dysplastic.

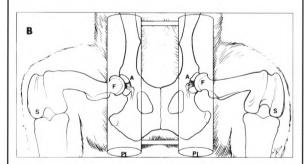
Using Labrador Retrievers, we have been comparing the new method with the standard leg-extended position. Adults considered normal when examined by the standard procedure showed femoral head displacements ranging from 1 to 3 millimeters when the new method was used. Hip joints rated as dysplastic by the standard method showed displacements ranging from 3.5 to 8.0 millimeters in the new procedure.

The most provocative findings, however, were in pups four to six months old. All had normal hips according to the conventional X rays, yet the pups fell into two groups when examined by the new procedure. One group showed displacements of 1 to 3 millimeters, the same as normal adult dogs; the other group, however, showed greater hip joint laxity with displacements of 4 to 6 millimeters (see illustration).

These observations suggest that while some pups appeared normal on the standard X rays, they in fact had abnormal displacements and may later become dysplastic. Although these preliminary results are promising, the pups must be observed for several years to determine whether those with greater joint laxity are, indeed, more susceptible to hip dysplasia than their counterparts with relatively tight joints.



A. Drawing of the conventional pelvic X ray taken with the dog's legs extended. According to that X-ray method, the hip joints are normal. Note that the femoral heads (F) fit snugly in the acetabula (A).



B. Drawing of a pelvic radiograph in which the same dog was positioned with its legs perpendicular to the tabletop. An adjustable plastic instrument (PI) was placed between the thighs, and inward pressure was applied at the stifle joints (S). The maneuver displaced the femoral heads (F) from the acetabula (A), revealing joint laxity that was not evident in the conventional X ray. The round ligament, which is not visible in a radiograph, joins the femoral head to the acetabulum.

We have been observing differences between normal cartilage from healthy canine joints and cartilage from dogs with osteoarthritis.



Nancy Burton-Wurster

Osteoarthritis

In other work in the laboratory, we have been observing the metabolic and biochemical differences between normal cartilage from healthy canine joints and cartilage from dogs with osteoarthritis, a disease which afflicts many animals, including dogs, horses, and humans.

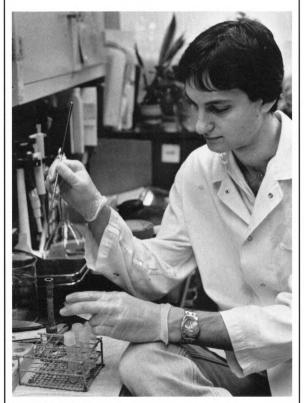
Although various differences between healthy and osteoarthritic cartilage have been observed, two are of particular interest. Osteoarthritic cartilage contains an accumulation of the glycoprotein fibronectin and a loss of proteoglycan, a structural element of the cartilage matrix. By using methods to differentiate among fibronectins from plasma, cartilage, and synovial fluid, we have concluded that the increased fibronectin does not come from plasma but is produced locally within the joint. At least some of it is made in the cartilage.

Preliminary evidence suggests that cartilage may contain a form of fibronectin found in very few other tissues. If so, this form of fibronectin may play a special role in maintaining the health of cartilage and in interacting with other components of the tissue, such as proteoglycan and collagen. By better understanding these roles, we hope to learn how the accumulation of fibronectin is related to the osteoarthritic process.

Studies conducted with other species, including humans and horses, reveal metabolic changes in cartilage that are similar to those observed in dogs. In collaboration with Dr. Robert Brown of Stanmore, England, we are conducting biochemical and electron-microscopic studies of canine and human cartilage from normal and diseased joints. Dr. Rory Todhunter, a graduate veterinarian from Australia, is examining the effects of selected drugs on cartilage repair in joints of horses.

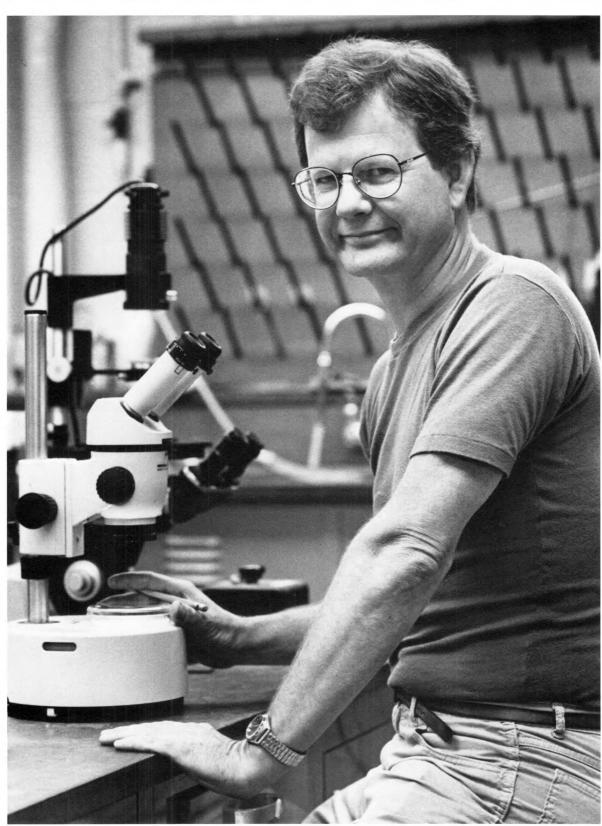
We are also characterizing and quantifying various aspects of cartilage metabolism using an *in vitro* method developed in our laboratory. Dr. Juergen Steinmeyer, a postdoctoral fellow who received his earlier training in Germany, is attempting to determine whether mechanical stress alters cartilage metabolism in ways similar to those observed in tissue from osteoarthritic joints. He is using an instrument developed by Dr. Peter Torzilli at Cornell Medical College. Using this device, controlled static and intermittent compressive forces up to ten times the body weight can be applied to cartilage.

George Lust Nancy Burton-Wurster



Harry Leipold

Our findings indicate that the gut is capable of initiating protective responses in areas that do not contain organized lymphoid tissue.



Robin Bell

Our work this year showed that lymphocytes triggered in an intestinal infection originate in the intestine and that populations of lymphoid cells throughout the intestine are capable of initiating and mounting their own local immune responses against invading pathogens. Previously it was believed that many immune responses in the gut were launched in collections of organized intestinal lymphoid tissue called Peyer's patches.

Immunity in the intestine, as in other tissues, depends on the purposeful interaction of various immune cells, including lymphocytes, macrophages, eosinophils, and mast cells. Some of these cells are generated locally, but many are recruited from the blood. To develop ways to boost the immune response of animals, it is important to learn where these cells are formed, how they reach the intestine, and how they interact in that tissue.

Much of our work has been with the infectious multicellular parasite Trichinella spiralis, which causes trichinosis. Last year we reported that lymphocytes, which have a helper function in immune responses, are released into the blood in substantial numbers for approximately thirty-six hours beginning about three days after an animal has eaten a meal containing T. spiralis larvae. Although these cells were known to enter the blood from the thoracic duct, it was not known whether the cells originated in lymph nodes draining the intestine or in the intestine itself. The answer was important because it would help determine certain functions of the cells and provide the initial step in understanding the stimulatory processes.

To find the answer, we surgically removed the mesenteric, or intestinal, lymph nodes from a group of rats before infecting them with *T. spiralis*. We found that this made little difference in the number of T-helper lymphocytes (those that bind antibodies that recognize distinctive molecules on the cell membrane) in the thoracic duct. Intestinal lymph nodes, therefore, are not a significant source of helper lymphocytes in this infection. These lymph nodes are also unnecessary for the expression of the immune response, for their removal did not impede the development of strong immunity against *T. spiralis*.

Immunity is triggered elsewhere in the intestine shortly after infection. Since helper lymphocytes divide at an early stage of the immune response, we enumerated dividing lymphoid cells in three different areas of the gut: the intestinal epithelium, the underlying lamina propria, and Peyer's patches.

To our surprise, we found that cell division increased twenty- to thirtyfold in the first two locations but not at all in Peyer's patches. Our findings indicate that the gut is capable of initiating protective responses in areas that do not contain organized lymphoid tissue. Our next step is to characterize these protective cells more precisely.

We suspect that the same cell interactions that initiate local immune responses against *T. spiralis* may be similar in a variety of other diseases. Chronic inflammatory bowel diseases, for example, are often associated with vigorous cell proliferation in the lamina propria.

We believe that the findings described above afford an opportunity to examine naturally induced immunity in the lamina propria and in the epithelium of the gut.

Robin G. Bell

Understanding how the immune system protects against infectious agents is the first step toward developing better vaccines.



Judith Appleton

Intestinal and respiratory tract infections take a great toll on animal health and result in significant economic loss. Understanding how the immune system protects against infectious agents that cause diseases such as trichinosis and influenza is the first step toward developing better vaccines and other methods that offer long-term protection against these debilitating diseases.

Trichinosis: A Model for Intestinal Immunity

Last year we reported that antibodies in rats previously infected with *Trichinella spiralis*, a multicellular parasite that causes trichinosis in carnivorous animals, will protect the animals and their suckling young from further infection. This year we have learned that not all classes of antibodies immunize the pups.

Both an antibody's class (its structure and functional characteristics) and its specificity (where it binds to a foreign molecule) are important. We have discovered that for antibodies to offer protection against trichinosis, they must bind to critical sites on the surfaces of the parasitic worms; antibodies that bind to a different part of the same antigenic molecule will not protect.

In related work, we found that when parasites are expelled from the intestine, they are entrapped in mucus. Yet that entrapment is not necessarily the most essential feature of protection; it appears to be secondary to the attack by antibodies. Dr. Melissa Carlisle, a graduate student in our laboratory, has shown that parasites already established in the intestinal tissue can be forced out by antibodies administered after the infection has developed. This implies that to offer protection, antibodies need not interact with the parasite as it enters the intestine from the stomach.

Our findings raise important questions about the function of the parasite's surface molecules against which the antibodies are directed and underscore the importance of isolating and characterizing those antigens.

Equine Influenza

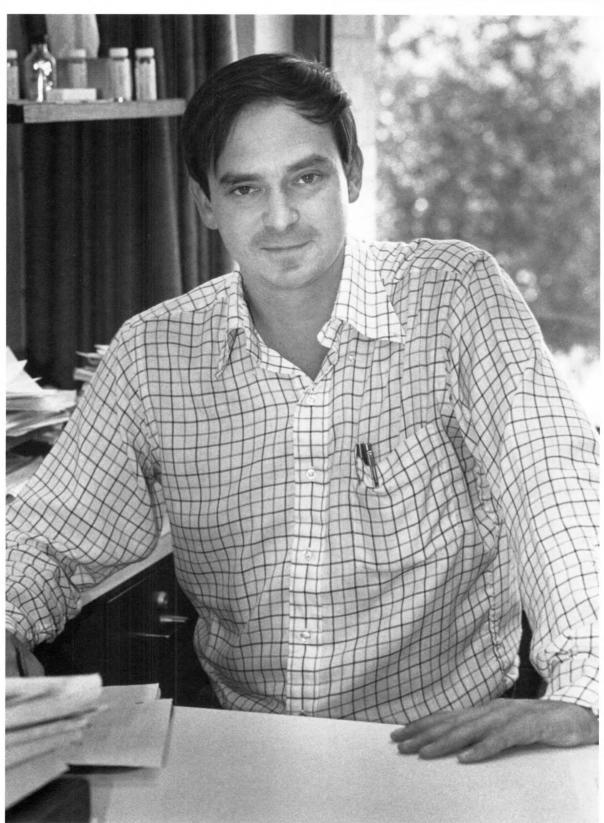
Current equine influenza vaccines fail to produce long-lasting protection. We have previously reported that this ineffectiveness is not caused by the invading viruses mutating over time; in fact, we have shown that the two major strains are relatively stable when compared with their human counterparts. The inefficiency of equine influenza vaccines, therefore, cannot be ascribed to the strains used in commercially available products.

To better understand the poor immune response of the horse, we have developed a method to isolate individual cells that produce virus-specific antibodies. It allows us to determine the exact specificity of equine antivirus antibodies and may reveal whether there are important sites on the virus that are not attacked by the antibodies. The roles of various cells of the immune system in producing antibodies are also being investigated.

These studies will help to elucidate how the immune response is regulated, and particularly whether the antibody response is turned off before strong, longlasting protection can be obtained.

Judith A. Appleton

During the Equine Genetic Center's past decade, our studies of reproductive physiology have expanded.



Douglas Antczak

Highlights Marking the Center's Tenth Anniversary

Our laboratory for equine studies at the Baker Institute was established a decade ago to study the major histocompatibility complex (MHC), a genetic region which controls several aspects of immune function in horses. Since then our studies have expanded to related aspects of reproductive physiology. Some highlights from the past decade include:

- Production and characterization of alloantisera defining Class I and Class II MHC antigens.
- Establishment of a breeding band of horses selected for their MHC types. These animals represent a unique resource, allowing many studies that could not be performed otherwise.
- Renovation of a historic barn complex, renamed the McConville Barn, in recognition of the generous support of Mrs. Dorothy Havemeyer McConville for this project. The College of Veterinary Medicine was awarded a certificate of merit from Historic Ithaca, a local landmark-preservation society, for the restoration work.
- Establishment of the first laboratory for monoclonal antibody production at Cornell and production of antibodies to a large number of equine cell surface molecules.
- Production of pregnancies in sterile mules and cross-species equine pregnancies by embryo transfer. This work has been conducted as part of a longterm collaboration with the British Thoroughbred Breeders Equine Fertility Unit, directed by Dr. W. R. "Twink" Allen.
- Organization of a continuing series of international workshops and symposia,

- sponsored by the Dorothy Russell Havemeyer Foundation on various topics in equine medicine.
- Establishment of a summer research fellow program for veterinary students, which is also sponsored by the Dorothy Russell Havemeyer Foundation. Two students are awarded fellowships each year for independent study at the Baker Institute.

Trophoblast Cells and the Expression of MHC Molecules

During the past year, graduate student Bill Donaldson continued his work on the expression of MHC molecules by the placenta. He devised methods that should facilitate the development of an *in vitro* model of trophoblast differentiation in the horse.

Previously, Dr. Donaldson had discovered that only the specialized and invasive trophoblast cells of the chorionic girdle region express MHC molecules, and that they do so only between days 30 and 45 of pregnancy. Taking advantage of the relative ease with which chorionic girdle cells can be grown in artificial media, Dr. Donaldson began to investigate the expression of MHC antigens by trophoblast cells in culture. The *in vitro* model will allow him to use drugs and chemicals to elucidate the cellular mechanisms that girdle cells use to switch off production of MHC antigens.

In the meantime, Dr. Julio Oriol, also a graduate student, has produced a monoclonal antibody that reacts with all forms of horse trophoblasts but with no other fetal, placental, or adult tissues. The unique expression of the target molecule by the trophoblasts suggests that it has an important function in early pregnancy and that it might be useful as an antigen for an experimental anti-fertility vaccine.

We expect to gain insights into complex immunoregulatory mechanisms.

Monoclonal Antibodies to Equine Lymphocyte Antigens

Chong-Hui Zhang, a graduate student from the People's Republic of China, has been producing and characterizing monoclonal antibodies to equine lymphocyte antigens with the goal of generating antibodies that can identify lymphocyte subsets of the horse. Such reagents will be used to characterize complex cellular reactions, such as the accumulation of maternal mononuclear cells around endometrial cups in normal horse pregnancies. Although the identity and function of these lymphocytes are not yet known, they represent the most striking example of a cellular immune response to the developing fetus described in any species.

As we unravel the significance of this response, we expect to gain insights into the complex immunoregulatory mechanisms that allow mothers to carry to term off-spring that express foreign paternal histocompatibility genes.

Sarcoid: Equine Skin Tumors

In other work, the genetic and virological aspects of the common equine skin cancer known as sarcoid were studied by John Angelos, a graduate student working jointly in this laboratory and with Dr. Carmichael in the Giralda Laboratory for Canine Infectious Diseases. Retrospective epidemiological studies revealed that Standardbred horses are highly resistant to sarcoid while Thoroughbreds and Quarter horses are quite susceptible. Mr. Angelos has also shown that virtually all sarcoid tumor tissue contains DNA that is closely related, if not identical, to bovine papilloma virus DNA. We are currently trying to identify equine MHC genes that could account for the differences among breeds in sarcoid susceptibility.

A Library of Horse Genes

While on sabbatical leave at the British Agricultural and Food Research Council's

Institute of Animal Physiology in Cambridge, I developed a library of horse genes using recombinant DNA technology and DNA isolated from the senior stallion at the Equine Genetics Center. This stallion is homozygous for genes in the equine MHC and, therefore, a very useful donor for these studies.

Through the use of special strains of bacteriophage (viruses that infect bacteria) that can continue to function when long stretches of their DNA molecules are removed and replaced with equivalent lengths of DNA from other species, a library of about one million recombinant bacteriophage was developed. Each bacteriophage contains a different segment of horse DNA. Having screened the library for bacteriophage that contain equine MHC genes, I am now beginning to characterize the genes I selected. These genes will eventually be used to study the mechanisms that control MHC gene expression in the horse placenta.

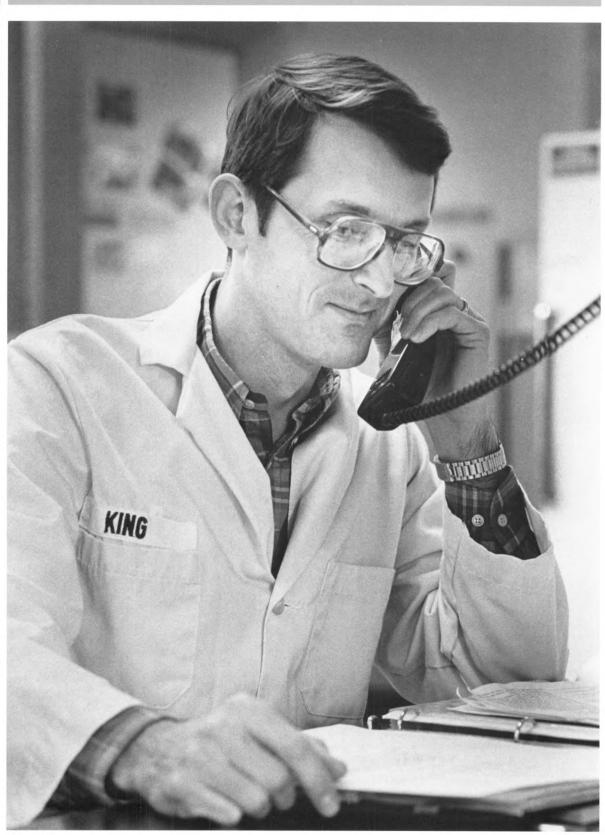
The "gene jockeys" at the Equine Genetics Center are off and running!

Douglas F. Antczak



Lorelei Dyess

If cytotoxic cells contribute to the host's defense against infection, it is mainly due to thymusdependent lymphocytes.



Alan King

Donnelley Laboratory of Immunochemistry

Certain bacteria have evolved in ways that enable them to thrive in their infected hosts' cells. Once the bacteria are protectively enveloped within their intracellular habitat, antibodies are powerless against the infection. While macrophages are the only cells capable of destroying intracellular parasites, they need signals from other cells, particularly thymus-dependent (T) lymphocytes, to promote their local recruitment and activation.

When rats are infected with the intracellular bacterium *Listeria monocytogenes*, thymus-dependent lymphocytes that have protective properties can be found in the animals' blood or thoracic duct lymph. Previously, we reported that the lymph of such animals also contains cytolytic cells—those with the capacity to attack and destroy other cells, including the ones harboring *Listeria* organisms. Yet the identity of these killer cells and their relationship to lymphocytes with protective properties remained a mystery.

Since several types of lymphocytes can destroy other cells but attack in different

ways, we set out to determine which kind are present in the lymph of *Listeria*-infected rats, and whether they have protective value. The principal candidates were T lymphocytes and so-called natural-killer (NK) cells.

This year, veterinarian Alan King, who is pursuing doctoral research in our laboratory, demonstrated that rats could be severely and specifically depleted of cytotoxic (cell-destroying) T lymphocytes while retaining normal levels of NK cells. The T-cell-deficient rats were vulnerable to a *Listeria* infection, and lymphocytes obtained from them had absolutely no protective value when transferred to normal rats.

This finding implies that NK cells have little, if any, role in acquired resistance to listeriosis. We conclude that if cytotoxic cells contribute to the host animal's defense against infection, it is mainly, if not exclusively, due to T lymphocytes.

Douglas D. McGregor

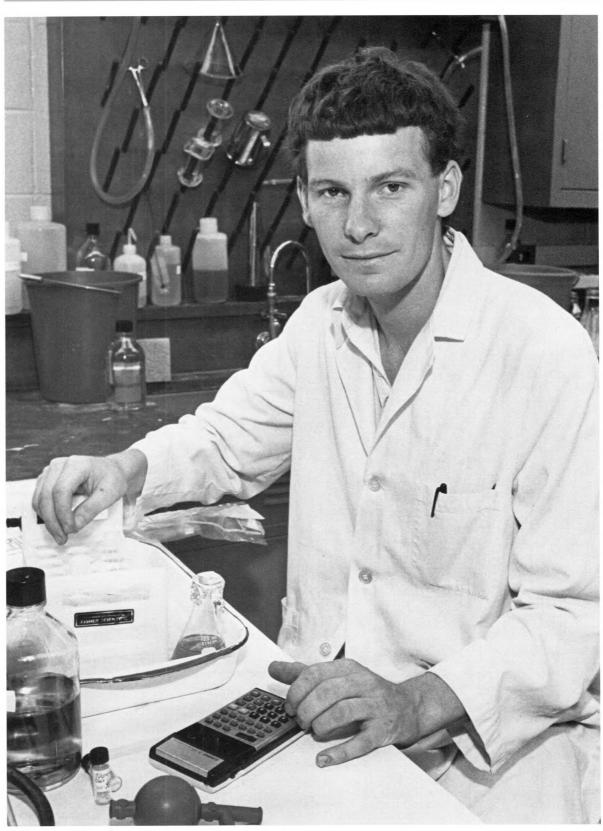


Lucille Gagliardo



Paul Lutwak

We are working to organize and create central resources that will facilitate the performance of experiments using molecular biological methods.



Colin Parrish

How do viruses, one of the simplest life forms on earth, evolve and adapt to new hosts? What changes allowed canine parvovirus type 2 (CPV-2) to emerge suddenly in 1978 and rapidly become a deadly threat to dogs worldwide? How can recombinant-DNA technology be applied to develop novel vaccines for CPV-2 and feline panleukopenia virus (FPV)?

These questions underpin our research, which seeks to define the relationships between the molecular structures of CPV-2 and similar viruses of cats and mink and their capacity to cause disease and adapt to new host species.

Viruses that infect animals, including humans, have well-defined host ranges. The individual parvoviruses that infect dogs, cats, and mink do not cause disease in the other species. Using the molecular biological techniques of DNA cloning and sequencing, and by analyses with antiviral monoclonal antibodies, we have shown that only very small differences exist between the viruses.

The evidence obtained suggests that CPV-2 evolved from FPV or mink enteritis virus (MEV), which were the causes of disease in cats or mink for years before CPV-2 burst on the scene. It also appears that a small region of the genome that codes for part of the virus coat protein is responsible for several unique properties of CPV-2, including its ability to replicate in dogs and its unique antigenic characteristics. Just a few mutations in this critical region appear to explain how CPV-2 derived from either the cat or mink virus. As the virus adapted to dogs, it lost its ability to replicate in its former host. The studies we are conducting are expected to reveal the molecular basis of that change.

In 1979 and 1980, a new variant of canine parvovirus emerged in the American dog population. This variant, CPV-2a, differs from the original type of CPV-2 in both antigenic characteristics and DNA

sequence. In collaboration with researchers in Japan, Denmark, Australia, and Washington State, we have shown that CPV-2a is now distributed globally and has replaced the original type of virus, except in "closed" kennels where exposure to "new" viruses is limited. Clearly, CPV-2a has a strong selective advantage over CPV-2, although the basis of that advantage has not yet been defined.

By analyzing sequence differences in the genomic DNA of CPV-2 and CPV-2a, we will attempt to define how specific differences contribute to the behavior of the viruses and try to identify which sequence differences and determine the selective advantage of CPV-2a over CPV-2.

In collaboration with Dr. Hussni Mohammed of the Department of Clinical Sciences in the College of Veterinary Medicine and Dr. Charles Aquadro of the Section of Genetics and Development in the Division of Biological Sciences, we are studying the evolution and variation of CPV isolates collected over the years from Japan, Australia, Europe, and the United States. We expect to gain new insights not only into the emergence and selection of CPV-2 but also into how CPV-2a evolved from the prototype CPV-2.

So far, all attempts to develop commercial vaccines against CPV or FPV using recombinant-DNA technology have failed. We are, however, examining some novel expression systems, including one in which the coat protein of CPV or MEV is expressed in an insect virus (baculovirus) that makes large amounts of the viral protein.

The molecular biology laboratory is also assisting other investigators at the Baker Institute by organizing and creating central resources that will facilitate the performance of experiments using molecular biological methods.

Colin R. Parrish

Publications

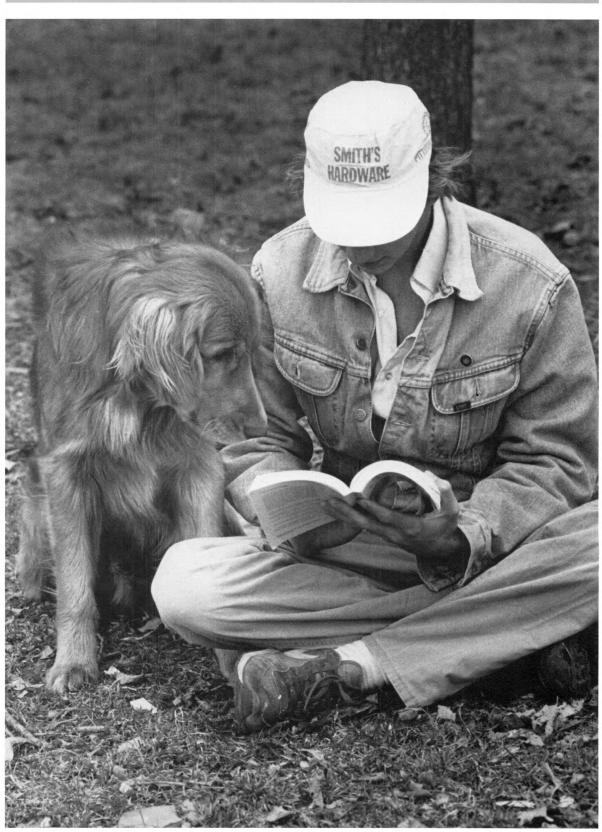
The following is a list of manuscripts published by staff members of the Baker Institute in 1988.

Publications listed as in press in last year's report are repeated this year, with their original numbers, to record their full bibliographic details.

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- **753 Appel, M. J. G.** 1988. Does canine coronavirus augment the effects of subsequent parvovirus infection? *Vet. Med.* April:360–66.
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- **767 Leipold, H. R., R. L. Goldberg,** and **G. Lust.** In press. Canine serum hyaluronate and keratan sulfate concentrations: Relationship to age and osteoarthritis. *Arth. and Rheumat*.
- **Parrish, C. R., C. W. Leathers, R. Pearson,** and **J. R. Gorham.** 1987. Comparisons of feline panleukopenia virus, canine parvovirus, raccoon parvovirus, and mink enteritis virus and their pathogenicity for mink and ferrets. *Am. J. Vet. Res.* 48:1429–35.
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Although dogs and horses continue to be the immediate beneficiaries of research at the Baker Institute, our findings touch on our own lives in immediate and palpable ways.



As you glance through this report you will detect an atmosphere of optimism and appreciate the depth and sophistication of the research now being conducted at the Baker Institute. The progress made this year can be ascribed in part to a continuing emphasis on health problems that have been a traditional concern of Institute scientists and also to the capacity of Institute staff to use resources in new and creative ways.

Dogs and horses continue to be the immediate beneficiaries of research conducted at the Institute. The findings described in our report, however, have wider relevance; they touch on our own lives in immediate and palpable ways. The reasons are not far to seek. Animals and humans are subject to many of the same diseases. That reality explains why so much of the research conducted at the Institute is sponsored by the National Institutes of Health. But the Institute is unusual and exceedingly fortunate inasmuch as it also enjoys the support and encouragement of a large and growing number of individual benefactors. Those individuals, either directly or collectively through dog clubs and other associations, have provided the margin of excellence and contributed in a meaningful way to the many discoveries that have emanated from the Institute over the past few decades. Our report honors those individuals and acknowledges their participation in our continuing effort to ensure happier, healthier lives for animals while simultaneously enriching our own.

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of Buttons)

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Mr. Ronald P. E. Leeds

of Jason)

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Mr. John M. Patterson

memory of Lilly)

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of Holly)

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memory of Misty)

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H. R. Sukhiani)

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