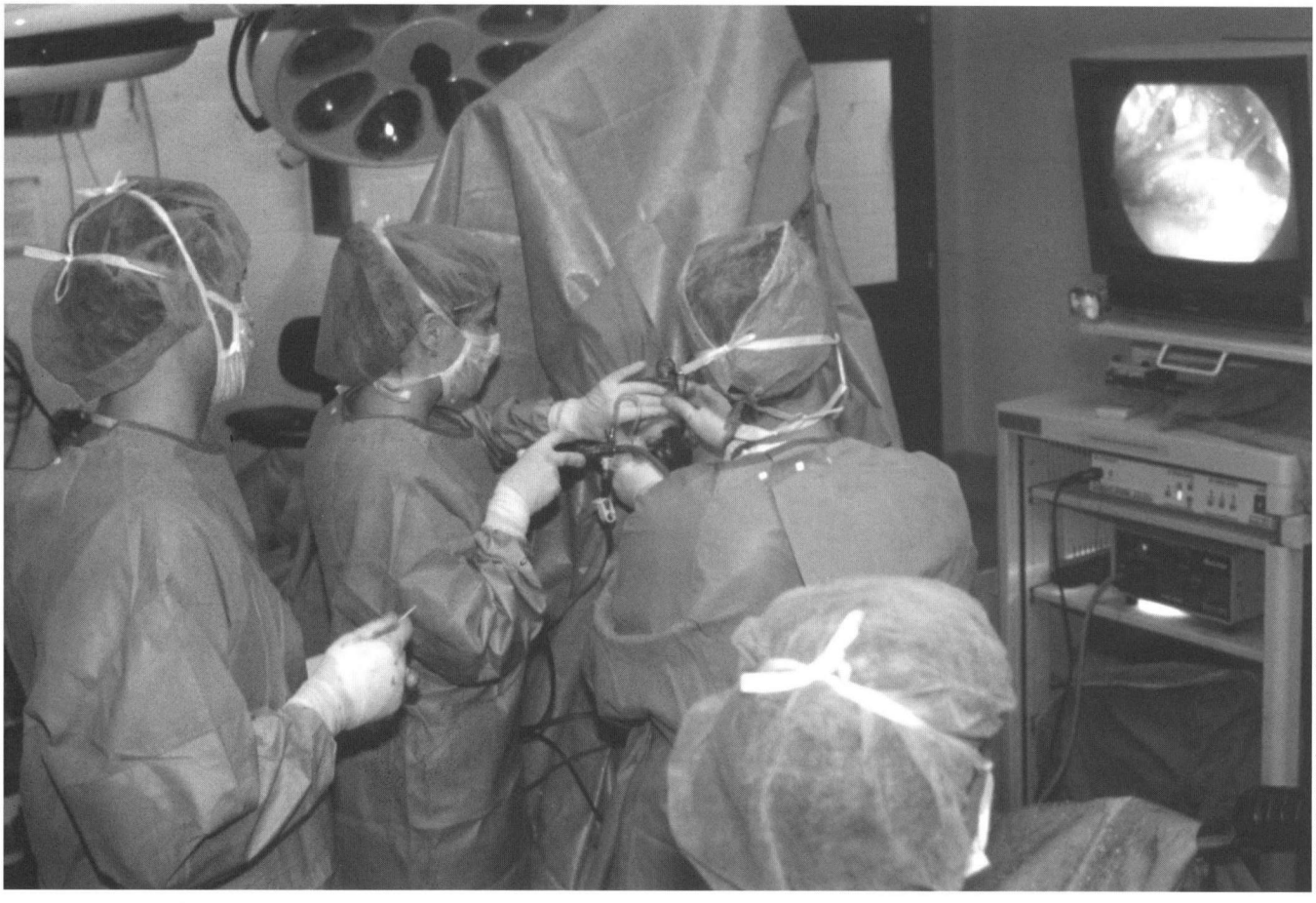
Zweig

Memorial Fund News Capsule

No. 31 July 2001

CORNELL

A report from the Harry M. Zweig Memorial Fund for Equine Research at the College of Veterinary Medicine at Cornell University



CHARLES HARRINGTON/UNIVERSITY PHOTOGRAPHY

Using minimally invasive arthroscopic surgery, Alan Nixon removes dead cartilage and bone and guides the injection of new growth factor–stimulated cartilage cells into the fetlock joint. The Thoroughbred yearling trained well and races for the first time this month.

INSIDE

Long-Lasting Gene Therapy Prevents Arthritis

Cartilage injury, which often can lead to osteoarthritis, is a major cause of debility among young athletic horses. Osteoarthritis frequently limits a horse's use, causes chronic disability, and sometimes even necessitates euthanasia. To help heal damaged joints and minimize or reverse arthritic changes in traumatized joints, Alan Nixon BVSc, MS, Dipl ADVS, an equine orthopedist and director of the Comparative Orthopedics Laboratory, is continuing to improve gene therapy for repairing equine cartilage damage. ▶

Gene therapy

continued from page 1

In his previous work, largely supported by grants from the Harry M. Zweig Memorial Fund, Nixon has successfully cloned a substance called the insulin-like growth factor (IGF-I), which is important for cartilage maintenance in healthy joints. He did so in collaboration with Christopher Evans, a professor of orthopedic surgery at the Center for Molecular Orthopaedics at Harvard University School of Medicine who is an expert in using gene therapy for treating arthritis in humans. Together they cloned IGF-I because it stimulates chrondrocyte cells to help build new cartilage inside a deteriorating joint. Nixon found IGF-I effective for promoting cartilage growth and the matrix synthesis of the cartilage surface in horses.

In 1998 and 1999, Nixon's work showed that by modifying adenoviruses IGF-I could be inserted into chondrocytes; the researchers found they were able to incorporate the genetically engineered IGF-I gene inside the adenovirus vector backbone. The modified adenoviruses are capable of penetrating living cells without causing any harm and deliver IGF-I DNA into the host-cell genome. Nixon calls the process transfection to distinguish it from infection, which implies having made a disease. Nixon found that this process could result in the production of active IGF-I protein for as long as 30 days.

In fact, largely because of this work, composites of IGF-I and chondrocytes are now used at the equine hospital at the Cornell University Hospital for Animals to repair cartilage and help improve joint regeneration. But the impact of IGF-I on the activity of transplanted chondrocytes is only short term.

Nixon's work in 2000 confirmed that the equine IGF-I gene could be injected into the horse's fetlock and seed the joint's synovial lining. Evidence of the IGF-I gene expression was still found in joint fluid 90 days later.

Recent work by Nixon also has shown that after cartilage injury, the joint shows an early deficiency of IGF-I, which then peaks at eight weeks, only to decline again beginning at 16 weeks and thereafter.

By inserting the gene for IGF-I, which stimulates chondrocyte metabolism, it is hoped that the healing substances will continue to be produced month after month, much like a manufacturing pump inside the joint, to promote cartilage repair and minimize or reverse arthritic changes in traumatized joints.

"This means there's an early and a late window of opportunity when administering supplemental IGF-I may be particularly useful for improving cartilage repair," says Nixon.

With a renewed grant from the Zweig Fund, Nixon and Evans are examining the effects of inserting the gene for IGF-I into chondrocytes just as they're being transplanted into the joint. They are optimistic that this will extend the impact of IGF-I beyond the four weeks previously reported. By inserting the gene for IGF-I, which stimulates chondrocyte metabolism, it is hoped that the healing substances will continue to be produced month after month, much like a manufacturing pump inside the joint, to promote cartilage repair and minimize or reverse arthritic changes in traumatized joints.

The 2001 grant funds an experiment with eight horses. The researchers will compare how cartilage defects in the horses' stifles compare when they are either filled with chondrocytes that have been enhanced with the IGF-I gene or with chondrocytes exposed to a null gene (the control). The first stage of the experiment was to produce more adenoviral IGF-I compounds.

The second stage, which the researchers are completing this summer, involves implanting the adenoviral IGF-I transfected chondrocytes into the horses. In the final stage, they will compare the healing process with arthroscopic examinations and biopsies at one and two months after implants and then will conduct a tissue analysis eight months after the transplants. They will determine how persistent the IGF-I gene is at each point and whether direct injections of IGF-I gene vectors to the joint fluid would be necessary in clinical cases.

"This dual approach builds on our ability to treat not only generalized joint disease by stimulating the production of new cartilage over long periods of time using direct gene therapy approaches, but also specific cartilage injuries with gene-enhanced chondrocyte implantations," explains Nixon. "Both approaches diminish the likelihood of arthritis and may possibly reverse the early stages of arthritis in horses and other animals." ■

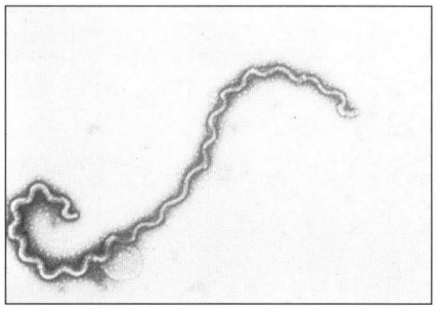
Watch Out for Leptospirosis

Leptospirosis is a growing problem for horse owners because there's no vaccine specifically for horses. As a result, leptospirosis can unpredictably infect horses and trigger fever, blindness, abortions, and even death. To prevent problems, horse owners are advised to keep an eye out for possible signs of leptospirosis and to take precautions to reduce the risks of infection.

Horses can get this bacterial disease, which also infects humans, cattle, wildlife, and most other animals, through the direct splashing of urine from infected animals (or from animals carrying the disease) into their eyes or from eating water, hay, or grain that's been contaminated by the urine of infected wildlife such as raccoons, mice, deer, or livestock. The bacteria can enter through direct contact with blood, urine, or tissues from infected animals; through the mucous membranes of the eyes, nose, or mouth; or through a cut in the skin. Not all horses that pick up the bacteria become ill, but if the immune system can't fight the infection, the bacteria can generate a lot of damage.

Leptospirosis respects no geographical or environmental boundaries. In recent years cases have been reported in horses in New York, Pennsylvania, and Kentucky. No one actually knows how many cases arise each year, although researchers do know that most horses in the Northeast, for example, test positive for having been exposed to leptospirosis.

If infected, a horse initially may develop a low fever for several days, lose its appetite, and seem dull or listless. Horse owners often first notice the disease when their horse develops painful-looking eyes caused by muscle spasms that close the eyes (severe blepharospasm), eyes sensitive to light (photophobia), eye discharge, tearing (lacrimation), squinting, puffiness around the eyes, eye cloudiness, or redness around the lids.



COURTESY OF YUNG-FU CHANG

The bacteria Leptospira interrogans *can enter a horse's body through direct contact with blood, urine, or tissues from infected animals; through the mucous membranes of the eyes, nose, or mouth; or through a cut in the skin.*

In addition to eye problems, leptospirosis can cause pregnant mares to abort. In fact, recent research shows that leptospirosis is one of the most important emerging causes of abortions in horses. Leptospirosis also can lead to decreased milk production, kidney failure, and death.

Diagnosing the infection is not always straightforward because no specific clinical signs are distinctive to leptospirosis although many of the symptoms do suggest the disease. Only laboratory tests can confirm a diagnosis. Prompt treatment, which may include steroids, antibiotics, and medications to dilate the eye, can relieve pain and minimize the chances of partial or total blindness. The horse may be more comfortable if it is kept in a dark stall or wears a fly-mask to protect against sunlight, dust, and insects.

Although leptospiral vaccines are available for farm animals and dogs, some outbreaks do still occur, perhaps because of vaccine inefficiency and/or because animals get infected by different *Leptospira* species than the vaccines protect for. With so many different *Leptospira* serotypes, no cross protection among them, and no vaccine available for horses, it's difficult to control or eradicate the disease. The vaccines for cattle are sometimes used in horses, but they don't protect against all the strains that may infect horses and can trigger side-effect reactions.

To better protect horses, researchers such as Yung-Fu Chang DVM, MS, PhD, DiplACVA, a professor of population medicine and diagnostic sciences, are working toward developing an equine vaccine. Chang, with a grant from the Harry M. Zweig Memorial Fund, is developing a genetic (DNA) vaccine or a recombinant vaccine against equine leptospirosis. He also is studying why *Leptospira* cause eye disease in horses, seeking to identify the protein(s) from *Leptospira* that can make antibodies against the equine eye tissues and cause autoimmune disease.

"It is very important that we develop a leptospiral vaccine that does not produce antibodies against normal horse eye tissues," Chang says.

Although horses don't easily spread leptospirosis to humans, the bacteria is contagious from animals to humans. Horse owners should assume that urine from any infected animal is potentially infectious and take the appropriate steps to protect themselves. People also can pick up leptospirosis from contaminated waters (such as ponds), direct contact with infected farm animals or rodents, or the blood or urine of infected animals, including pets. The disease is rarely fatal for humans.

To protect horses, owners should keep their animals from drinking stagnant water, water in marshy areas, or water from ponds that could have been contaminated by wildlife or cattle. They should practice good management of manure (both water and manure can harbor the spirochete), fence in water sources to keep wildlife out, drain wet muddy areas where horses are pastured, and disinfect any areas where infected animals may have been. ■

EPM Continues to Puzzle Scientists

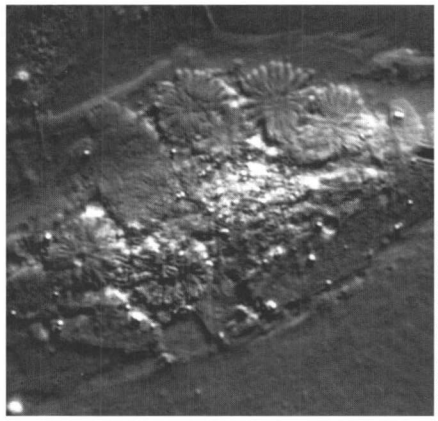
The diagnosis of equine protozoal myeloencephalitis, or EPM, is a chilling moment for any horse owner. EPM, which is infectious, is now the most common neurological disease afflicting horses, accounting for more than 35 percent of all equine spinal cord disease in the United States. It can also affect the brain and brain stem.

EPM's most common signs are incoordination and weakness, but the initial signs may be low key and more vague, such as mild lethargy, attitude change, subtle gait changes, inability to exercise as well as before, and difficulty maintaining complex gaits. The more severe signs include incoordination, inability to stand, lameness, facial paralysis, stumbling, falling, and tremors. Although most common among young adult horses and yearlings in training or young racehorses, the disease can infect horses of any age.

EPM, which is becoming more and more commonly diagnosed, can be life threatening. Veterinary scientists are still puzzled over many aspects of the disease, says Dwight Bowman MS, PhD, an associate professor of parasitology, who has been studying the disease since it was identified in 1991.

"Veterinarians are perhaps more puzzled now than they were just a few years ago when they thought the various pieces had come together to explain the method by which horses became infected and why disease developed," he says.

In the late 1970s, researchers found a microscopic protozoan in the nervous tissue of horses with signs of neurologic disease. In the early 1980s, it was suggested that these organisms were similar to the *Sarcocystis* group of parasites that characteristically have two hosts: a final carnivorous host in which the parasites live in the intestinal mucosa and produce transmission stages that are passed in the feces, and an herbivorous intermediate host that ingests the fecal stage



COURTESY OF DWIGHT BOWMAN

Rosettes of the protozoan organisms that cause EPM can be grown inside cells under laboratory conditions.

and develops cysts in their tissues. In the early 1990s, the parasite was isolated from horses that had developed severe signs of EPM, and the isolated organism was given the name *Sarcocystis neurona.*

To find the host of the disease, researchers looked at potential final hosts whose geographical distributions overlapped those of the reported cases of EPM. EPM, they found, followed the distribution pattern of the common opossum, and indeed, using molecular tools, the opossum was identified as the natural final host of this organism. When researchers went to the literature, they matched the newly identified *Sarcocystis neurona* with one described a hundred years ago as *Sarcocystis falcatula.*

"This led to a fairly well-grounded and acceptable hypothesis that the EPM organism was *S. falcatula* and that it was cycling between a sarcocyst stage (cysts in the muscle tissue) in birds and a sporocyst stage spread throughout the environment in opossum feces," Bowman explains. "In this scenario, horses are infected by eating or drinking material contaminated with sporocysts shed by opossums."

Several newer studies, however, including one conducted at Cornell, have shown that feeding uninfected horses up to 60 million sporocysts from the feces of infected opossums does not cause the signs of the disease or any immunological changes in the horses' blood. In some cases, horses fed sporocysts have developed neurologic signs, but neither organisms nor the DNA of organisms have been recovered from these horses at necropsy. Also, it has been recently shown that the organisms isolated from horses are morphologically different from those considered to be the avian parasite *Sarcocystis falcatula.*

"Thus it appears that the initial jubilation over having discovered the source of equine infection has been misplaced and we find ourselves almost exactly where we were when the organism was first described in 1991," says Dr. Bowman. "Now the possibilities seem twofold. One possibility is that the actual identity of the species of *Sarcocystis* cycling in wildlife has not been determined and, therefore, the experimental infection of horses does not induce disease. A second possibility is that some other factor or agent predisposes horses to develop disease or causes an existing infection with *Sarcocystis neurona* to change from a latent to a lethal condition."

Bowman recently received a grant from the Harry M. Zweig Memorial Fund to obtain information relative to the first of these two possibilities. The hypothesis continues to be that the causative organism in EPM sheds sporocysts in the feces of an animal that is indigenous to the Americas because EPM has not been observed in Europe, Africa, or Asia. He also believes that cysts that serve to infect these carnivores are present in muscles in small vertebrates such as birds or rodents.

"We know there are many species of *Sarcocystis* in wildlife that are either not described or very poorly known. Basically, we will reproduce the work that led to the choice of the opossum-bird model with other carnivore

EPM

continued from page 4

and omnivore-prey systems," explains Bowman. "Although we still do not know what the source of infection is for the horse, we now have a much greater ability to look for it than we did in the early 1990s," he says.

Bowman's laboratory now has the expertise to maintain *Sarcocystis* species in culture. He also has a bird model in the parakeet that allows easier isolation of organisms in cell cultures and the development of muscle cysts that can be fed to potential final hosts. His lab has the technology to distinguish the various *Sarcocystis* species molecularly, and work is progressing collaboratively with Dr. Antoinette Marsh at the University of Missouri and Dr. J. P. Dubey at the U.S. Department of Agriculture in Beltsville, Maryland.

> "Our goal is to isolate and identify an organism from wildlife hosts that is identical to the EPM organism we find in diseased horses and is capable of inducing the disease in horses."
>
> Dwight Bowman

"Our goal is to isolate and identify an organism from wildlife hosts that is identical to the EPM organism we find in diseased horses and is capable of inducing the disease in horses. Work by others and ourselves has indicated that the opossum is host to several *Sarcocystis* species, and one of these may be *Sarcocystis neurona*."

Bowman plans to isolate various *Sarcocystis* sporocysts from the feces of wild carnivores such as opossums, raccoons, skunks, foxes, and coyotes and, perhaps, some raptors and vultures that will be used to infect parakeets and mice. From the parakeets and mice, Bowman and his colleagues will harvest organisms to initiate their growth in cell culture. Since each carnivore probably is a host to more than one species of *Sarcocystis*, the researchers will try to develop techniques to identify specific isolates.

Treatment and Prevention

Thomas Divers DVM, Dipl ACVIM, a professor of medicine who is the coinvestigator on this project, has been wrestling with the treatment of this disease for years.

"Once horses become infected with the culprit species of *Sarcocystis*, probably by ingesting grain or other food or drinking water that has been contaminated, the parasites migrate to the central nervous system, including the spine and brain," he says.

Recent studies have shown that about 50 to 60 percent of horses in areas with high rates of EPM, such as the Midwest and Northeast, seem to have been exposed to *Sarcocystis*, though only a small percentage actually develop neurological symptoms. Researchers believe that symptoms develop from the body's inflammatory response to the parasite causing structural damage in the central nervous system.

The disease is difficult to diagnose without a full battery of tests that includes a spinal fluid sample to look for antibodies against the parasite. Early treatment is important for recovery. Treatment, which typically lasts one to four months, involves parasite-killing drugs and sometimes anti-inflammatories and immune system-boosting medication. While most horses improve with treatment, only about 10 to 20 percent of treated horses seem to make a full recovery. About 70 percent partially recover, and about 10 percent must be euthanized. If left untreated, most horses tend to deteriorate.

"At present, the best way to prevent this disease is to protect horses from exposure to potential sources of feed contaminated with the feces of wildlife," Bowman says. "Try to keep opossums, raccoons, and other carnivores out of horse barns. Keep grain for horses in containers with tight-fitting lids. It is important to keep watering troughs as clean as possible. It is of course impossible to prevent infection when animals are at pasture, but maintaining a tidy pasture with minimal trash or surrounding undergrowth will be likely to preclude the activity of most of these hosts." ■

Harry M. Zweig Memorial Fund for Equine Research—2001 Awards

The following research awards were made by the Zweig Committee at its annual meeting in November.

Renewals

$38,790 to Dr. Dorothy Ainsworth for "Attenuating Exercise-Induced Immuno-Suppression in Athletic Horses"

$51,695 to Dr. Douglas Antczak for "Horse Genome Project"

$51,717 to Dr. Yung-Fu Chang for "Equine Leptospirosis: Part I. Vaccination against Equine Leptospirosis in a Hamster Model"

$46,055 to Dr. Kevin Haussler for "Vertebral Movements and Applied Forces During Spinal Manipulative Therapy in Horses: A Randomized Controlled Study"

$65,818 to Dr. Alan Nixon for "Growth Factor Gene Enhanced Chondrocyte Transplantation for Equine Cartilage Repair"

Revised

$73,430 to Dr. Yung-Fu Chang for "Equine Lyme Disease: Antibiotic Treatment of *B. burgdorferi* Persistent Infection"

Total Zweig funds awarded—$327,505

The Harry M. Zweig Memorial Fund for Equine Research honors the late Dr. Harry M. Zweig, a distinguished veterinarian, and his numerous contributions to the state's equine industry. In 1979, by amendment to the pari-mutuel revenue laws, the New York State legislature created the Harry M. Zweig Memorial Fund to promote equine research at the College of Veterinary Medicine, Cornell University. The Harry M. Zweig Committee is established for the purpose of administering the fund and is composed of individuals in specified state agencies and equine industry positions and others who represent equine breeders, owners, trainers, and veterinarians.

2000 Harry M. Zweig Memorial Fund Committee

Jean Brown
Blue Chip Farms, Inc., Wallkill, NY

Bruce Hamilton
Executive Secretary, Harness Horse Breeders of New York State Albany, NY

John L. Hardy
Featherstonhaugh, Conway, Wiley & Clyne, LLP, Albany, NY

Mike Hoblock
Chairman, NYS Racing and Wagering Board, Albany, NY

John E. Jagar, DVM
Millbrook, New York

Philip Trowbridge
NYS Thoroughbred Breeding & Development Fund Corp., Ghent, NY

Charles Knauss
Executive Director, Agriculture and NYS Horse Breeding Development Fund

Paul C. Mountan, DVM
Rhinebeck, NY

Donald F. Smith, DVM, Dipl ACVS
Dean, NYS College of Veterinary Medicine at Cornell University Ithaca, NY

Harry D. Snyder
Saratoga Springs, NY

Patricia Wehle
Wehle Stock Farm, Scottsville, NY

William Wilmot, DVM
Saratoga Springs, NY

Anna Zweig
Middlebrook Farm, Nassau, NY

CORNELL

Zweig News Capsule
College of Veterinary Medicine
Cornell University
S3020 Schurman Hall
Ithaca, NY 14853-6401

The Zweig News Capsule is produced periodically throughout the year to report on research at the College of Veterinary Medicine, Cornell University, funded by the Harry M. Zweig Memorial Fund for Equine Research.

Writer: Susan S. Lang
Editor: Metta Winter
Designer: Barbara Drogo
Photography: Cornell University Photography, Dwight Bowman, Yung-Fu Chang

6/01 GPP 3.5M MTS00549

Printed on recycled paper

Cornell University is an equal opportunity, affirmative action educator and employer.

Produced by Media and Technology Services at Cornell University

www.mediasrv.cornell.edu