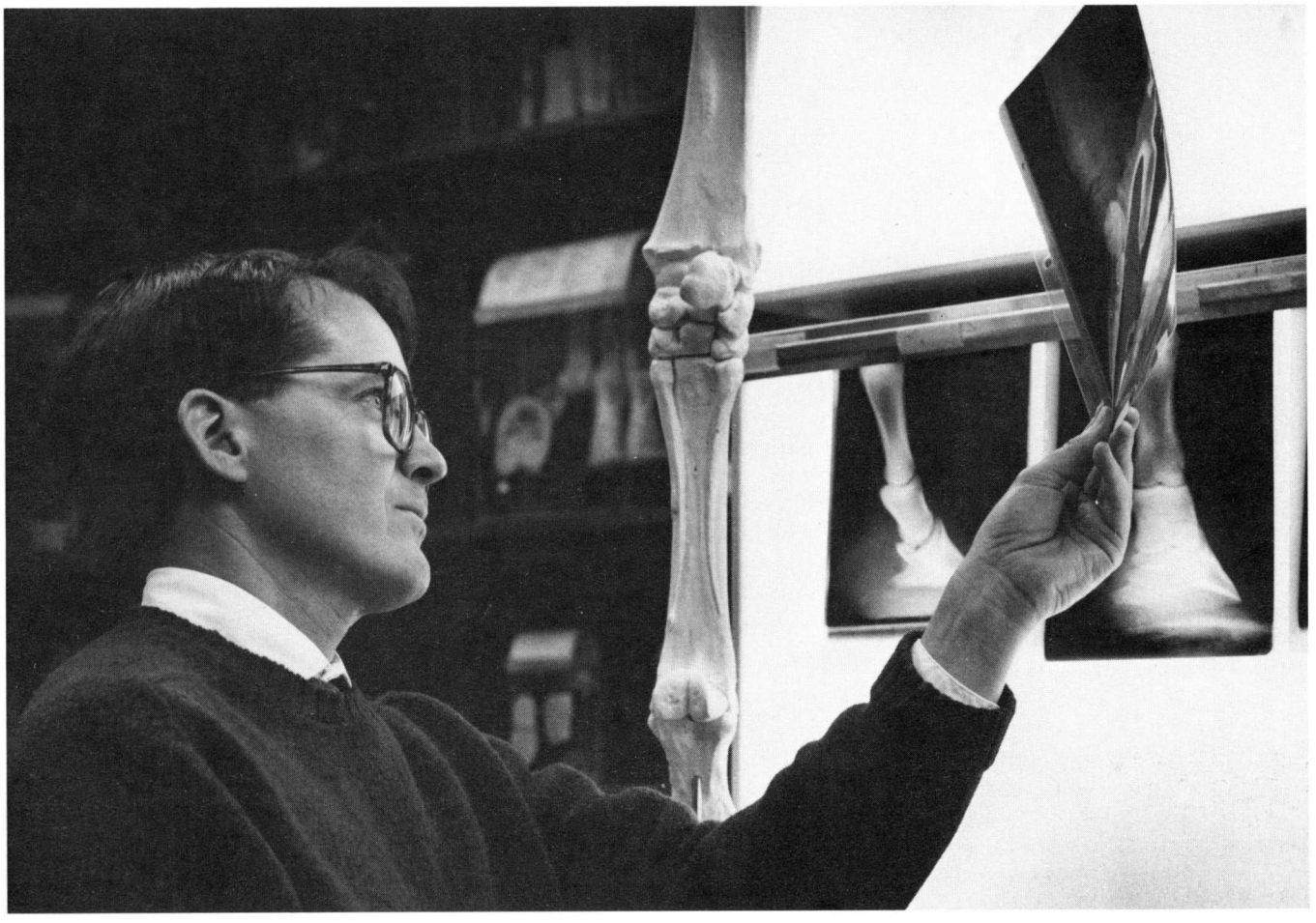
Zweig

Memorial Fund News Capsule

A report on equine research at the College of Veterinary Medicine at Cornell sponsored by the Harry M. Zweig Memorial Fund

No. 12 May 1992



BRUCE WANG

INSIDE

Common Protein Found in Shoulder Muscle and the Diaphragm

Combats lameness and premature foal deaths

For the first time in a mammal, a form of protein found in fetal and neonatal muscles also has been found in muscles of the forelimb of adult horses. Furthermore, the research done by Dr. John W. Hermanson (above) has located these same forms of myosin in the neonatal diaphragm, information that could be helpful for saving premature foals. ▶

Previously, it was thought that this biochemical form of myosin, a major protein involved in muscle contraction, was only present in the muscles of fetuses and neonates before the muscles matured.

The discovery has its roots in Hermanson's research into the biology of the biceps brachii, the muscle that comes over the horse's shoulder joint. Hermanson, a zoologist and expert in locomotion, was intrigued when he first observed a triplet pattern of the fetal myosin isoforms (types) in the lateral head of the biceps brachii.

"The discovery grew increasingly interesting as we found the same triplet pattern of fetal isoforms—so-called slow native myosin isoforms—in muscles down the leg, specifically in the superficial digital flexor and the 'slow' compartment of the deep digital flexor," said Hermanson, whose research is supported by the Harry M. Zweig Memorial Fund.

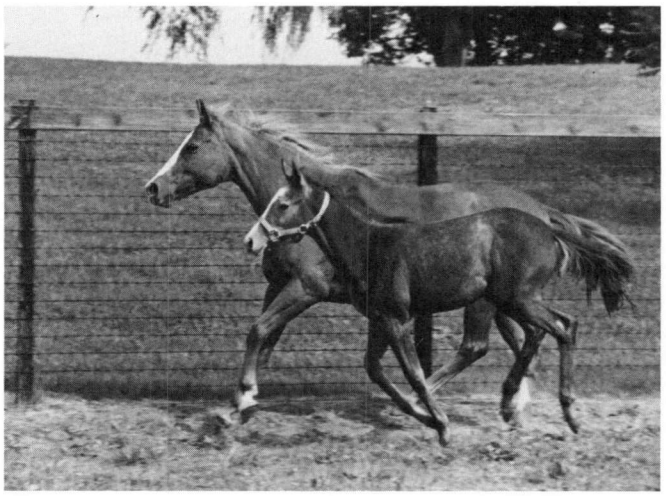
Hermanson noted that the isoforms were found only in muscles that perform particular functions—fatigue-resistant, repetitive, postural activities. These include the muscles that participate in the passive-stay apparatus, the non-tiring mechanism that allows horses to stand at birth and upright for 90 percent of their time without growing excessively tired.

Fatigue-resistant forelimb muscles are of vital importance to athletic horses, and Hermanson and veterinary student Matthew Cobb have been fine-tuning descriptions of their biology.

By chance, while working on a collaborative project with a pediatric neonatal specialist in Pittsburgh, Hermanson observed the triplet pattern of slow native myosin isoforms in the diaphragm of a premature foal. Further investigation showed that this pattern was typical in neonatal diaphragms.

"This discovery made sense since the diaphragm, an essential muscle of respiration, is required to maintain repetitive contractions for the entire life of the animal, without signs of fatigue and without using extra muscle energy," Hermanson explained.

"We now suspect that in the evolution of the horse, the retention of these neonatal biochemical muscle conditions is related to the neonate's ability both to generate normal breathing movements and to maintain a constant normal standing posture throughout life—both fatigue-resistant mechanisms."



CHARLES HARRINGTON

John Hermanson's study of proteins may lead to better rehabilitation programs for horses.

Fatigue-resistant forelimb muscles are of vital importance to athletic horses

He said the triplet pattern is not known in any other adult mammalian muscle, yet he suspects he will continue to find it in other equine muscles used in repetitive activities but not required to produce great force.

Finding the triplets of slow native myosin in foal diaphragms has been a major turning point in Hermanson's research. Respiratory failure is a major cause of death in equine newborns during the first two weeks of life, and attempts to sustain neonates on ventilators have proved largely unsuccessful.

"We suspect that the diaphragm is still in an immature state in premature foals. When it gets pushed mechanically by a ventilator or becomes stressed by other extrinsic factors, such as a nervous system condition, the muscle can be easily destroyed."

Hermanson seeks to document the normal, functional transitions of fetal and neonatal isoforms in the development of specific appendicular muscles and the neonatal diaphragm, identified by changes in the underlying biochemical and histochemical states. He hopes to determine the chronology of these sequences and to correlate the presence or absence of slow myosin isoforms with specific functions in the maturation of muscles. In the case of the diaphragm, he is interested in identifying critical periods in development that would indicate when or how mechanical ventilators could be used or should not be used. This information would help veterinarians do a better job in keeping premature foals alive.

Hermanson also plans to test his hypothesis that deviation from a specific sequence of developmental isoforms puts an animal at higher risk of lameness, or death in the case of diaphragm failure.

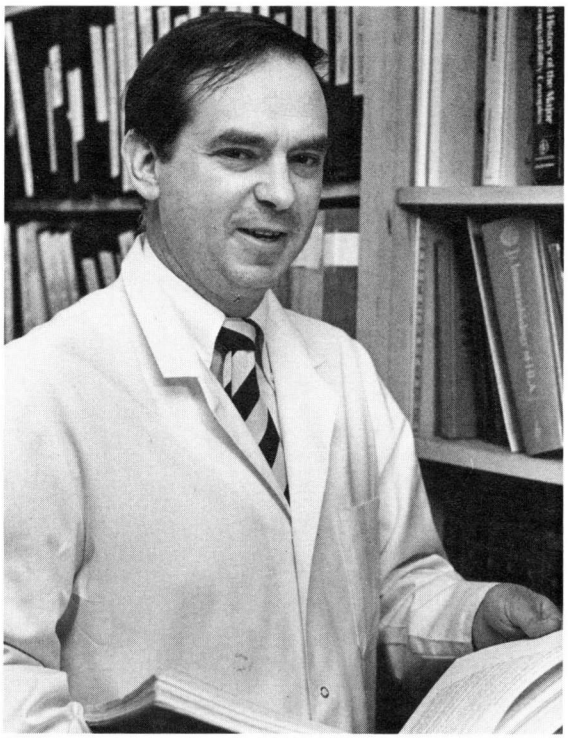
"By better understanding how the muscular system of horses is organized during ontogeny, and how these isoforms are affected by extrinsic factors, it may be possible to design improved therapeutic measures to assist horses through the crisis of premature delivery and to design better rehabilitation programs for horses suffering from athletic injury." ■

Why Mares *Don't* Reject Embryos

Research has meaning for transplants and tumor research

Most organ grafts made between unrelated individuals are promptly rejected by the recipient's immune system, unless the donor and recipient are carefully matched for tissue compatibility (also called histocompatibility) genes or the recipient's immune system is suppressed by drugs. Successful outcome of most mammalian pregnancies is much different. Although embryos contain foreign genetic material from the father, maternal immune systems usually don't reject them, even though mother and embryo are not matched for tissue compatibility, and the mother is not immunosuppressed. A better understanding of how the successful pregnancy 'graft' is established and maintained is important for several medical areas:

- Undesired abortions in horses and perhaps in humans
- Rejection of skin grafts and organ transplants
- Destruction of tumors that "hide" from their hosts' immune systems



CHARLES HARRINGTON

Immunologist Douglas Antczak and his group have conducted one of the first studies of molecular genetics in horses.

Dr. Douglas Antczak, a veterinary immunologist who heads the Equine Genetics Center, focuses much of his research effort on the immunogenetic aspects of early placental development in the horse.

Antczak had previously identified which cells in the trophoblast (the outermost layer of the placenta that forms a barrier between the mother and fetus) express paternal MHC (major histocompatibility complex) antigens during fetal implantation and which don't (*News Capsule* No. 4, 1989). Class 1 MHC produce antigenic cell surface molecules that largely determine whether foreign tissue will be accepted by the host or not. The researchers had found that while trophoblast cells that burrow into the uterine lining express high levels of paternal MHC antigens, the class 1 MHC genes in the non-invasive trophoblast cells are somehow turned off and stop producing antigens. The same thing happens in the endometrial cups in the placenta — temporary ulcer-like structures that secrete an important pregnancy hormone.

"By these mechanisms, the developing fetus 'hides' its paternally inherited MHC antigens and avoids immunological destruction by the mother," Antczak explains.

Antczak and his research group have isolated class 1 MHC genes from different trophoblast cells to determine the structure of the molecular "switches." Using recombinant DNA techniques, Antczak, Dr. Dina Barbis and Dr. Julie Maher, a Ph.D. student in immunology, have been the first to successfully sequence and clone DNA of equine class l MHC genes.

"This effort constitutes one of the few studies in equine molecular genetics underway at any institution worldwide," Antczak says. "We've been able to determine precisely which MHC genes we have identified and are being expressed. This is not an insignificant problem. The horse, like most mammals, carries 20 to 30 closely related class 1 MHC genes, and we're interested in only one or two of them. The specific antibodies and genetically selected horses we've assembled over the past decade are invaluable aids in this work."

Having identified which genes are being expressed, the researchers' next step is to dissect the genes' promoter and suppressor regions—the part that turns them on and off—to determine how they are transcribed in particular cells and to analyze any difference between the proteins from the cells that turn genes on and the cells that turn genes off in the trophoblast.

Antczak and his colleagues have developed two libraries of expressed genes made from horse lymphocytes (white blood cells) and from equine trophoblasts. Each contains about 50,000 specimens. The research group is isolating the class 1 MHC genes from the different forms of trophoblast cells to determine the structure of the molecular "switches."

Antczak also expanded his arsenal of molecular tools when he co-organized the First International Workshop on Leucocyte Antigens on the Horse last summer in the United Kingdom. Researchers at the workshop shared their panels of monoclonal antibodies that help them identify different types of horse leucocytes and lymphocytes.

Using these new tools, the Equine Genetics Center Group has probed deeper into understanding the maternal lymphocyte attack that occurs around the endometrial cups in the placenta. They are now better equipped to define the types of lymphocytes that inhabit the uterus and their function in pregnancy.

"With these new cell surface markers for lymphocytes in the horse," Antczak says, "we'll be able to better understand the immune response to any virus or bacteria under study and to develop improved methods for diagnosis and treatment of equine disease involving the immune system." ■

Robin Gleed

BRUCE WANG

Robin Gleed makes a notation on instrumentation chart used in respiratory research.

"Point-to-point racing," known as steeplechase racing in the United States, was a favorite pastime around the dairy country of Gloucestershire, England, where Robin Gleed grew up. Although he never owned horses himself, whenever he could Gleed would ride the horses on whatever dairy farm he was working on at the time, or he'd escape to the races with his friends.

During high school Gleed realized he could combine his interest in dairy cattle with his passion for horse racing. "I remember when I'd be working on a farm, a cow would get milk fever or have trouble during birth. A local vet would come around, and just fix it," said 38-year-old Gleed. "It might be bloody, but it was glamorous to me and left quite an impression."

That impression fueled Gleed to do well enough during high school to go straight on to veterinary college at the University of Liverpool ("High schools in Britain cover a lot more territory than they do here in the states," Gleed says.). By 23 he was a veterinarian, but he stayed on to study veterinary anaesthesia for two more years. "I'd always been interested in the problems of the respiratory system."

Dr. Gleed first came to Cornell as a visiting assistant professor in 1978. He met a young American woman in charge of the supplies at the animal hospital and they married. The Gleeds returned to Britain, this time to private practice in Boroughbridge, North Yorkshire, to work exclusively with race horses involved in national hunt racing.

A job offer from Cornell forced him to compare the options of coming back to Ithaca to teach and do research vs. endlessly driving around the English countryside as a working vet. Ithaca won out.

As an associate professor of veterinary anesthesiology in the department of clinical sciences, Gleed spends about half his time in the clinic and operating room working on all kinds of animals. The remainder is spent teaching veterinary anaesthesia and doing research.

Now, rather than going to the races, Gleed does research on the respiratory problems that plague race horses, focusing currently on blood pressure in the lung and hypoxia (low oxygen). Horses, unlike other species, have a particularly low level of arterial oxygen when they exercise. Gleed and colleague Dr. Alan Dobson suspect that hypoxia in horses is a limiting factor during racing and exercising. They are studying whether the usually high blood pressure seen in the lungs of exercising horses leads to water accumulation in the lung. This water may, in turn, inhibit the rate at which oxygen can get into the horse through the lungs; they also suspect that Lasix will act to reduce this water accumulation. In an unrelated study, Gleed is studying the cardiovascular effects of Buscopan, a pain killer for horses with colic, which is not yet approved by the Food and Drug Administration.

In his free time, Gleed and his wife, Anne, are raising their five children—three boys and two girls—a dog and a cat, and are putting the finishing touches on a new house they moved into several months ago. And if there's any time left over, Gleed runs to keep in shape, helps out with soccer coaching at his children's school, and gardens to tame the mud around his new home. ■

John F. Cummings

DAVID GRUNFELD

The research of John Cummings crosses the boundary between horses and humans.

When 12-year-old John F. Cummings learned about an after-school job mucking out horse stalls from a casual chat with a friend, his fate was sealed. The stable was two bus rides away from his school, the pay was low, and the work was dirty, but he took the job anyway.

Before long, Cummings knew that veterinary medicine was to be his life work. He worked at the stable for three years, playing broomstick polo with empty beer cans when he could (which gave him experience to play on the Cornell freshman polo team) and worked summers on a farm.

Entering Cornell in animal science almost 40 years ago was Cummings's first step to becoming a Cornell blue blood. He earned his bachelor's, D.V.M., master's, and Ph.D. degrees at Cornell, met his wife here, sent four of his five children here (one transferred elsewhere), and has been a professor in the College of Veterinary Medicine for 25 years.

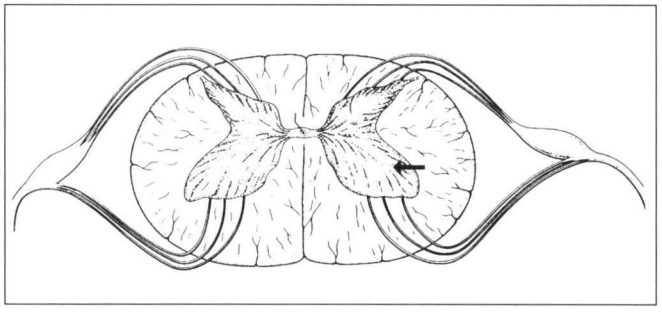
As a veterinary comparative neurologist, 55-year-old Cummings, with the help of Dr. Sandy deLahunta, has explored dozens of mysterious nervous system conditions that show up at the Large and Small Animal Clinics at Cornell, "As the comparative neurologist on the team there, my role has been to study how tissues are affected at the cellular level using the electron microscope," Cummings said. In many cases, the team has not only studied the animal disease at hand but has successfully developed animal models for studying closely related human neurological diseases.

For example, Cummings has studied coonhound paralysis, which is similar to the human condition of Guillain-Barre Syndrome; a muscle and spinal cord disease in Swedish Lapland dogs that resembles infantile spinal muscular atrophy; an auto-mutilation disease that causes English pointers to literally bite their feet off and has striking similarities to hereditary sensory neuropathy in humans; and a spinal disease in Afghan hounds resembling subacute combined degeneration in people.

"Many of the models are so-called storage diseases (e.g., Tay Sachs disease) caused by inborn errors of metabolism and occur early in life. Typically, nerve cells become backed up with lipids that cannot be degraded because of the absence of an enzyme. Often, the goal is to figure out which enzyme is missing and how to replace it."

Cummings has explored these diseases and related topics in some 80 scientific papers and a forthcoming text on veterinary neuropathology. In projects funded by the Harry M. Zweig Memorial Fund, Cummings, Gil Burns, and Sue Hackett have studied the innervation, or motor control, of equine intestinal walls, which he said are "unbelievably highly evolved but hadn't been studied before," as well as the underlying causes of roaring in horses (see *News Capsule* No. 7, 1990, for articles on both). Working with Dwight Bowman this past year, he described the electron microscopic features of the parasite that causes equine protozoan myelitis.

Cummings and the neurologic disease team are perplexed by a recently identified disease called Equine Motor Neuron Disease. The debilitating nerve disease of unknown origin closely resembles Amyotrophic Lateral Sclerosis, or Lou Gehrig disease, in humans. "So far, the pattern of cases has no rhyme or reason, but just like the other diseases we've studied, we'll keep narrowing down possible causes and testing them until we figure it out." ■



Spinal cord cross section. Arrow points to motor neuron area that degenerates in newly identified disease.

The Harry M. Zweig Memorial Fund 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 funds and is composed of individuals in specified state agencies and equine industry positions and others who represent equine breeders, owners, trainers, and veterinarians.

The Harry M. Zweig Memorial Fund Committee

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Harry M. Zweig Memorial Fund for Equine Research Awarded Projects in 1992

- $50,146 to Dr. Dorothy Ainsworth for "Respiratory Muscle Activity in the Equine Athlete and Its Role in Performance Limitation"
- $38,971 to Dr. Douglas Antczak for "Immunogenetic Studies of the Horse"
- $40,544 to Dr. Judith Appleton for "Analysis of the Equine Immune Response to Influenza Virus"
- $48,571 to Dr. Barry Ball and Dr. Joanna Ellington for "Co-culture of Gametes and Early Embryos in the Horse"
- $49,941 to Dr. Peter Daels for "Prolonged Luteal Activity in Non-pregnant Mares"
- $5,000 to Dr. Peter Daels, Dr. Joanne Fortune, and Dr. Robert Hillman for "Renovations at the Reproduction Unit of the Equine Research Park"
- $37,918 to Dr. Normand Ducharme and Dr. Richard Hackett for "The Effect of Furosemide on Athletic Performance in Race-horses"
- $8,586 to Dr. Normand Ducharme and Dr. Richard Hackett for "Supplemental Appropriation for Study of Respiratory Mechanics in Exercising Horses"
- $36,865 to Dr. Brian Farrow for "Support for Research Associate in Equine Sports Medicine"
- $47,806 to Dr. Joanne Fortune for "Regulation of Ovarian Follicular Development and Function in Mares"
- $39,140 to Dr. Robin Gleed and Dr. Alan Dobson for "Effect of Lasix on Capillary Pressure and Water in the Lungs of Exercising Horses"
- $16,120 to Dr. John Hermanson for "Muscle Morphology and Myosin Biochemistry in Equine Muscles: Necessary Transitions in Development"
- $16,920 to Dr. Harold Hintz for "Effect of Exercise Intensity and Duration on Oxygen Uptake, Metabolites, Heart and Respiratory Rates"
- $54,633 to Dr. Alan Nixon for "Chondrocyte-Laden Tissue Analogues for Transplantation Resurfacing of Extensive Cartilage Defects in Horses"

Total Zweig Funds Awarded $491,161

In the next Zweig News Capsule: A survey of your opinions.

Zweig News Capsule
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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: James P. Titus
Designers: Wendy Kenigsberg, Barbara Drogo
Photographers: Bruce Wang, David Grunfeld, Charles Harrington

6/92 MS 4.9M E11073N

Printed on recycled paper