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The Study of Abnormal Muscle Function in Horses

Jill Beech
University of Pennsylvania

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Abnormal muscle function is an important cause of exercise intolerance in both human beings and horses. In man, some myopathic conditions can be crippling. Examples are myotonic dystrophy and some of the "storage diseases" where metabolism is altered, and abnormal products accumulate in the muscle. Although a myotonic dystrophy-like condition does occur in horses, storage diseases have not yet been reported, and we are more commonly faced with muscle dysfunction that prevents the horse from exercising normally or performing his/her expected work. Another disorder of muscle function, hyperkalemic periodic paralysis, which occurs in both human beings and in Quarter Horses, can cause severe weakness, recumbency and paralysis and has been implicated as the cause of death in some horses. Nutritional deficiency of Vitamin E and selenium can cause myopathies in many animals, including the horse, as well as in man.

Some years ago a liaison was established between several clinicians at New Bolton Center and Dr. Henry Rosenberg's research group in the Malignant Hyperthermia Laboratory at Hahnemann University. That collaboration has flourished, and our investigation of muscle function and muscle disorders has expanded. My research has entailed close collaboration with Dr. J. Fletcher in this laboratory and also with Dr. S. Wieland at Hahnemann. In addition, Drs. Gronod and Mehta at Scott Ritchey Research at Auburn University collaborate on these studies.

The major dysfunction we have been studying is "tying up" or chronic intermittent rhabdomyolysis (CIR) or exertional rhabdomyolysis. The clinical term "tying up" refers to the appearance of stiffness and in severe cases inability to move. There are varying degrees of severity from a mildly impaired stiff or "poor" gait and slowing down with reluctance to continue exercise to marked generalized muscle stiffness, anxiety and distress, severe pain (occasionally to the point of being violent and simulating an acute abdominal crisis such as a twisted intestine) sweating, and difficulty walking.

In addition, the muscles themselves develop a "leak" in their membrane, and enzymes which normally reside in the muscle cell spill through the muscle membrane into the blood. Other intracellular substances such as myoglobin, which is important in carrying oxygen for the muscle, also spill out. It is the latter substance which, as it is cleared from the blood by the kidney, discolors the urine a dark red or port wine or coffee color in severe cases. To many horsemen this is a "red flag" for "tying up". However, although this grossly visible myoglobinuria is diagnostic for damaged muscle, its absence does not mean muscle damage or cramping has not occurred. We see quite a few horses that "tie up" yet have normal appearing urine.

More sensitive indicators of muscle damage and leak are the presence of "muscle enzymes" in the blood and the levels increase to a variable degree depending on the amount leaked. Although increased levels in the blood indicate abnormal muscle and muscle "breakdown", the elevation does not always correlate with the severity of clinical signs. Also, some elevation normally can be seen early in a horse's training program and in response to exercise. As these enzymes are cleared from the blood at different rates, measuring both of them helps one to identify when the horse had a problem or if the condition is continuing. Some horses may not overtly "tie up" but perform poorly and have abnormal increases in these enzymes. Horses may show signs even before they have really exerted themselves, i.e. in the early stages of exercise or when just walking out to the racetrack prior to work. More horses probably show signs during or after exercise, but the condition apparently is rare after an actual race.

Signs may be very intermittent and it may be impossible to identify a "trigger". Certainly diet can be a factor. When draft horses were in common use the condition was known as "Monday morning disease" as the horses showed signs on Monday following a day of rest on Sunday while being maintained on their regular "work day" heavy carbohydrate meals. Hormonal factors are probably important as the condition is reported more frequently in young female horses (fillies), and preventing estrus cycling sometimes eliminates the condition. Changes in exercise pattern, stress and genetic factors are all probably important. However, the exact cause and mechanisms for the disorder remain to be determined. Our studies suggest that abnormal muscle membrane function is one mechanism.

Another equine myopathy is very similar to myotonic dystrophy in human beings. Horses with a myotonic dystrophy-like syndrome may have grossly enlarged muscle groups (like weight lifters) and their gait is altered and progressively deteriorates. The horses I have known with the condition have all been euthanized at a relatively young age. Sharply tapping the muscle usually elicits a "myotonic dimple" or muscle contraction which is slow to relax. Some forms of myotonia, including myotonic dystrophy, in humans are inherited.

A third myopathy we have started investigating is hyperkalemic periodic paralysis (HPP). This condition is inherited and has been reported only in horses who are of the Quarter Horse breed or part Quarter Horse bred. Its onset may be as early as a few months of age. Horses have intermittent bouts of muscle trembling and weakness, contraction of the lips, abnormal movement of the third eyelid like in tetanus, and they sometimes go down and are unable to rise. At these times, their blood potassium is elevated. There is a potential for cardiac arrhythmias and these have been implicated as the cause of death in some of these horses. Affected horses have an abnormal muscle membrane; the "membrane potential" is less negative and is closer to depolarizing or "firing" than normal. A high potassium meal (for example: alfalfa hay or first cutting timothy hay, sweet feed with molasses) can trigger onset of signs because it alters the flux of ions across the muscle membrane. Exactly what it is in the membrane that results in this altered membrane, heretofore, has not been determined and studies of other ions important in contraction and relaxation of muscles also are needed.

Whenever we have a horse with a suspected muscle disorder we obtain as much clinical history as possible to try to determine potential triggers, when signs started, frequency, etc. Clinical laboratory data on blood electrolytes and muscle enzymes are recorded. In some select cases we may also run hormone levels to determine if a filly is cycling normally or if there is evidence of abnormal thyroid function. Although hypothyroidism can cause signs of myopathy, I have not yet found evidence of abnormal thyroid hormone levels in horses that ty up, and the horses show no other clinical signs of deranged thyroid function.

Abnormal electrolyte (ion) status or abnormal movement across membranes can affect muscle function. Our studies and those of Harris and Snow in Newmarket, England and also work in man have implicated abnormal potassium balance or passage across the muscle membranae (transmembrane flux) as a possible cause of tying up or CIR. Unfortunately, blood levels of electrolytes are not accurate indices of cell levels, especially for electrolytes such as potassium which is highest in cells and relatively low outside cells simultaneously. Measuring urine and blood levels of electrolytes and computing urinary excretion has been suggested to be a method of determining total body status or at least whether the horse is depleted; for example, if urine levels of potassium are lower than normal, one can assume the horse is trying to conserve it because of having insufficient intake and/or body stores. If a horse has abnormally low urine potassium or sodium, supplementation is initiated (and has been successful in selected cases as a treatment for CIR). However, one would like to determine whether this fractional excretion actually does accurately reflect cellular levels of these substances. The red blood cell (RBC) potassium has been reported to reflect body cellular (including muscle cell) levels but, to my knowledge, this has never been satisfactorily validated — certainly not for the horse. Therefore, we are measuring muscle cell potassium levels in those horses we biopsy and comparing these levels with the RBC and urine levels. S. Lindborg, the technician who plays a vital role in these studies, has been determining the best methodology for determining muscle potassium, and she is comparing levels in RBC and muscle. We are also investigating the effects of altered dietary potassium intake on these levels and we'll be able to correlate these with our simultaneous muscle function studies. In the racehorse population that is not available for biopsy, we are currently looking at urine fractional excretion and measuring red blood cell potassium levels in horses that are normal and in those with CIR to determine whether there is a correlation between the two clinical laboratory tests and whether horses with CIR differ from normal.

Surgical biopsies are obtained from the semimembranous muscle with the horse standing and sedated. This muscle is a large bellied muscle of the hind quarters comprised of approximately 90% fast twitch fibers and is the one we have used for our studies as it is important in locomotion. The alignment of muscle fibers allows dissection of muscle strips for contraction studies, and its large size allows multiple biopsies with no ill effect on the horse.

The section of muscle is divided for mechanical function studies, biochemical (including lipid) analysis, microscopic evaluation, including histochemistry, determination of potassium content, study of electrolyte channels in the muscle membrane, and assessment of calcium uptake and release in the inner muscle membranes involved with muscle contraction.

Briefly, for the muscle function studies, strips of aligned muscle fibers are dissected and placed in a muscle bathing solution that maintains health and viability of the muscle (and also allows addition of drugs and other chemicals to evaluate their effect). The pattern of contraction and relaxation and force generated can be measured. A section of muscle is processed for studying calcium release from the

components involved in muscle contraction and relaxation. A small piece of muscle is cultured so that new cells develop and the cell line can be maintained for a prolonged period, allowing long term studies and evaluation of ion (electrolyte especially potassium and sodium) currents into and out of individual cells. As we believe the cause of muscle dysfunction resides in the membrane (in CIR, HPP and myotonia) much of our work focuses on this. A section of muscle is evaluated microscopically for its structure and fibre type. The biochemical analysis is performed by chromatographic methods. A small section of muscle is analyzed for potassium content using spectrophotometry following special preparation methods.

To date we have found that a certain proportion of horses with CIR have prolonged muscle twitches that can be normalized by phenytoin (Dilantin, a drug that affects sodium and potassium movement across membranes and is most commonly known because of its use in epilepsy). Phenytoin is also effective clinically in stopping these horses from having CIR. Phenytoin also shortens the prolonged twitch of horses with myotonia and we are presently studying its effects in HPP. Horses with CIR also show other alterations in their muscle mechanics. Studies on calcium release and uptake and effects of various drugs are ongoing. Our initial studies showed a lower threshold for calcium release in horses with CIR. The muscle culture work and investigation of ion channels is unique to our studies. Most of the data on cultured muscle cells must still be analyzed. However, initial studies on the HPP muscle cells has shown an abnormal membrane current.

Biochemical studies have shown altered phospholipids in horses with CIR: these are major components of muscle membranes and this again supports altered membrane function. Histochemistry and histology have shown no altered fiber typing and often minimal light microscopic degenerative changes in horses with CIR, but horses with a MD-like syndrome had marked changes. Obviously, if a biopsy is taken when the horse is "tyed up" changes may be marked but these are probably secondary, not primary. This again supports the hypothesis of altered membrane dysfunction in CIR (and some of the changes in MD may be due to abnormal calcium leak and subsequent muscle necrosis).

Our studies on potassium are ongoing and will continue both in normal horses on diets with varying potassium, horses with CIR and those with HPP. We are expanding our in vitro studies to look at various other mediators. There are no reports on muscle cell culture and ion channels in any equine myopathies, and we're especially interested in pursuing these studies. Various other biochemical analyses are being performed. At Scott Ritchey, studies on red blood cell fluidity are being conducted on our horses as altered red blood cell membrane character has been associated with some myopathies in other species and if an association is found with certain equine myopathies, this could be a good noninvasive screening diagnostic tool. We are also studying the clinical aspects and various muscle characteristics of an offspring of 2 horses with the myotonic dystrophy-like syndrome. Our research has already generated substantial original information for presentation at various scientific meetings and I think our collaborative approach offers a very unique opportunity for studying muscle disorders.

Jill Beech, V.M.D.
Associate Professor of Medicine

Farm Show

The School's booth was bigger this year and in a new location. Faculty, staff and alumni manned it during the week-long event.

Student Equine Symposium

The University of Pennsylvania's student chapter of the American Association of Equine Practitioners held its fourth annual Equine Symposium on November 18, 1989. More than one hundred students attended including 19 visiting students from Cornell, Tufts, Ohio State, North Carolina State, and Virginia Polytech Institute.

The yearly symposium gives students the opportunity to learn more about specific areas of equine medicine and practice procedures to which they normally would not be exposed until their senior year. In addition, Penn's students can meet and become better acquainted with the clinicians at New Bolton Center.

For the morning session of the program, students attended three wet labs of their choice, which were taught by clinicians who volunteered their time. The labs provided hands-on experience in a variety of topics including the use of the YAG laser, techniques of arthroscopic surgery and internal fixation, neonatal intensive care, reproductive exams of the mare and stallion, neurologic and ophthalmologic exams, echocardiology, field anesthesia, the use of nerve blocks in lameness diagnosis, the use cytology in diagnosing respiratory, joint and abdominal disease, radiology, surgical approaches to colic, and the anatomy and surgical approaches to the limbs and upper respiratory tract.

Penn Veterinary School's alumni society sponsored the lunch for all participating students and clinicians which was followed by a talk by Dr. Sue McDonnell, Ph.D. Dr. McDonnell spoke on her work in stallion behavior.

The date for Equine Symposium V will be announced by the end of the school year. More information on this event can be obtained from Amy Hartman, the 1990 president of Penn's chapter of the SCAAEP.

Wildlife Report

1989 was another busy year for the Wildlife Service, receiving a record 395 cases. Our release/placement rate for the year was 45%. There are at present approximately 50 first and second year student volunteers who put in a great deal of their limited free time to help provide the best care possible to our patients.

We have been very lucky to have an increased interest in Wildlife by several people in Veterinary School community, especially Dr. David Thomson, who have donated their time to provide the utmost care for our injured animals. Subsequent to Dr. William Medway's resignation, Dr. Charles Newton has taken on the role of Wildlife advisor.

Some of our most interesting cases include:

- A fledgling Peregrine falcon, found under the Walt Whitman bridge, was brought to the Wildlife Service, was treated and placed into another nest in Philadelphia by the State Game Commission and the Peregrine Fund.
- A red tail hawk was referred from Tri-State Bird Rescue in Delaware with a fractured ulna and tibiotarsus due to gunshot injuries. After several surgeries to repair the tibiotarsal fracture and surgical treatment of a severe case of bumblefoot, the bird was rehabilitated and after 14 months in captivity was released at New Bolton Center.
- A mallard duck that was hit by a car was admitted with a fractured radius and ulna, cranial skin loss and 9 ducklings. All of the ducklings and the mother duck were eventually released.
- An immature bald eagle needing orthopedic surgery was admitted. The bird, which had been shot, was found outside the Blackwater Animal Refuge in Maryland. The legs of the eagle were broken and a bullet was lodged in the right leg. Dr. Gail Smith, associate professor of surgery, removed the bullet and stabilized the fractures with external fixation devices. The surgery lasted almost five hours. The bird was transported to Tri-State for long-term care and a more suitable environment to accommodate its seven-foot wingspan.
- This winter, Wildlife members assisted Dr. Olney Pierce in necropsy of several of the birds that died in the oil spill on the Arthur Kill in New Jersey.

The Wildlife Service is currently lobbying for renovations of the wildlife ward and outdoor facilities. We are also in the process of improving our selection of reference materials and obtaining more equipment to provide the best possible care for our patients.