The effects of non-focused extracorporeal shock waves on neuronal morphology, function and analgesia in horses

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THE EFFECTS OF NON-FOCUSED EXTRACORPOREAL SHOCK WAVES ON NEURONAL MORPHOLOGY, FUNCTION AND ANALGESIA IN HORSES

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
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In

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by

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LIST OF ABBREVIATIONS

CAP – compound action potential
CGRP – calcitonin gene-related peptide
cm – centimeter
DJD – degenerative joint disease
DMD – dorsal metacarpal disease
EHL – electrohydraulic lithotripsy
EMG – electromyography
ESW – extracorporeal shock waves
ESWL – extracorporeal shock wave lithotripsy
ESWT– extracorporeal shock wave therapy
FEI – Federation Equine International
G – ground electrode
H & E – hematoxylin and eosin
Hz – hertz
IV – intravenously
kg – kilogram
LFB – luxol fast blue
LWRL – limb withdrawal reflex latency
mJ – millijoules
mm – millimeter
MPa – megapascals
mV – millivolt
m/s – meters per second
µs – microsecond
nsec – nanosecond
R – recording electrode
S – stimulating electrode
SDF – superficial digital flexor
SNCV – sensory nerve conduction velocity
STIM – electromyographic stimulator
TEM – transmission electron microscopy
ABSTRACT

These studies were conducted to elucidate the regional analgesic effect that is observed clinically after treatment of orthopedic disorders with application of extracorporeal shock waves in horses. Regional analgesia after treatment with extracorporeal shock waves presents a concern because it may eliminate protective limiting mechanisms and may place equine athletes with predisposing lesions at risk of sustaining career- or life-ending injuries.

Direct percutaneous application of non-focused extracorporeal shock waves to palmar digital nerves in the pastern area of horses resulted in decreased sensory nerve conduction velocities compared with untreated control nerves at 3, 7, and 35 days after treatment. Transmission electron microscopy revealed distinct morphological changes consisting of extensive separation and disruption between the different layers of the myelin sheath in large- to medium-sized myelinated axons of treated palmar digital nerves.

Treatment of selected areas of the metacarpus in horses with non-focused extracorporeal shock waves failed to identify a regional analgesic effect when cutaneous sensation was assessed by comparing the nociceptive threshold (limb withdrawal reflex latency, LWRL) between treated and non-treated areas after stimulation with a focused light source. The LWRL responses in all horses were comparable in treated and control areas over time with a significant decrease noted at most sites and time points compared with baseline values.
CHAPTER 1

GENERAL INTRODUCTION

Musculoskeletal disorders are the most prevalent cause of wastage in Thoroughbred racehorses and other performance breeds. Wastage is a term used to describe either the loss of horses from conception to adulthood, or the days lost by equine athletes due to inability to train or compete (Olivier et al. 1997). Musculoskeletal disorders reportedly have a major financial impact on the equine industry, both in terms of loss of athletic performance of the horse and expenses for diagnostics and treatments (Kobluk et al. 1990).

Extracorporeal shock wave therapy (ESWT) represents a new treatment modality used to treat selected orthopedic injuries in horses and appears to be gaining considerable interest and acceptance among veterinarians, trainers and horse owners. Extracorporeal shock waves (ESW) were first used in humans in the 1980’s to fragment renal and urinary bladder stones (Chaussy et al. 1980). Since 1985, concretions in other organs such as gallbladder, bile duct, pancreas and salivary glands have been treated with ESW (Iro et al. 1989; Iro et al. 1992; Sauerbruch et al. 1986; Sauerbruch et al. 1987). In the past ten years, ESW were also successfully used to treat a variety of orthopedic disorders in humans, such as fracture non-unions, pseudarthroses, osteochondrosis and various tendinopathies (Haupt 1997). Based on these encouraging results, ESW were adapted to treat orthopedic disorders in horses.

Extracorporeal shock waves are acoustic pressure gradient waves with a rise time of 5 to 10 nanoseconds (ns) to a peak pressure of up to 100 megapascals (MPa), followed by a rapid fall to negative pressure, before returning to baseline in a total pulse time of
approximately 300 ns (Sturtevant 1996). Two fundamentally different techniques are used to generate ESW, focused and non-focused generators (McClure and Merritt 2003). Focused ESW generators initiate pressure waves within a fluid medium. These waves are focused by reflection within the generator and directed toward a focal point in the body of the patient (Sturtevant 1996). Non-focused ESW or radial pressure waves are generated by mechanical concussion and transmitted into the patient’s body by use of a hand-held applicator. They are characterized by lower wave energies, a slower rise time than focused ESW, and a negative component that is approximately of the same order as the positive component (McClure and Merritt 2003). There is no focusing mechanism and the maximum energy generated by non-focused ESW is observed at the applicator-patient skin interface and declines rapidly in proportion to the distance from the generator. Both types of ESW generators are currently used to treat orthopedic disorders in horses (McClure and Merritt 2003).

Although the exact mechanism by which ESW exert their effects in tissues is not fully understood, a transient local analgesic effect within shock wave-treated areas has been observed in humans and horses (Haupt 1997; McClure and Merritt 2003; McClure et al. 2003). Several painful soft tissue syndromes in humans, such as tennis elbow and golfer’s elbow, are currently treated using low energy ESW with considerable success (Haupt 1997). Local analgesia occurring after ESWT application is most likely independent of any other beneficial effect on tissue healing and could place equine athletes at risk of sustaining career-ending or life-threatening injuries, such as condylar fractures of the third metacarpus or metatarsus or breakdown of the suspensory apparatus of the fetlock, if exercised under the influence of local analgesia. These safety concerns
have been recognized by the equine industry and have recently resulted in development of regulations regarding the use of ESWT prior to training and competition by racing jurisdictions and the Federation Equine International (FEI). Due to the potentially catastrophic and fatal consequences of an analgesic effect that eliminates self-limiting protective mechanisms after ESWT application, side effects of this new treatment modality should be thoroughly investigated prior to widespread, uncontrolled application in performance horses.

The global hypothesis of this thesis was that ESW cause a transient local analgesic effect when applied to the distal limb in horses and that this effect is at least partially mediated by alterations in function and morphology of peripheral nerves within treated areas. To address these concerns, two studies were conducted.

The purpose of the first study was to identify functional changes in superficial peripheral nerves by measuring sensory nerve conduction velocities (SNCV) in palmar digital nerves directly treated with non-focused ESW compared with non-treated control nerves at different times after application. Additionally, it was intended to determine any relationship between changes in SNCV and morphologic changes in treated nerve segments by histology and transmission electron microscopy (TEM). It was hypothesized that SNCV in ESW-treated nerve segments would decrease, compared with non-treated control nerves, and that characteristic nerve lesions would be present after ESW application.

The purpose of the second study was to determine if a local cutaneous analgesic effect occurs after non-focused ESW application to the dorsal aspect of the third metacarpus in horses by using a standardized pain model and by comparing treated
versus non-treated control legs within the same animal. Additionally, it was intended to
determine the interval of time of altered nociception in treated legs after ESW
application. It was hypothesized that horses would exhibit an absent or delayed limb
withdrawal reflex latency (LWRL) in ESW-treated legs in response to a painful stimulus
with a focused light source. It was further hypothesized that the response to the pain
stimulus would not be different in treated and non-treated limbs after attenuation of the
local analgesic effect.
CHAPTER 2

REVIEW OF LITERATURE

2.1 Significance of Lameness in Performance Horses

Lameness represents an indication of a structural or functional disorder in the horse’s limbs or back that can be observed while the animal is standing or in motion (Stashak 2002a). Causes for lameness include trauma, congenital or acquired conformational anomalies, infections, metabolic disturbances, circulatory and nervous disorders, and various combinations of these (Stashak 2002a). Diagnosis and treatment of disorders causing lameness in horses are associated with considerable cost and have a major impact on the equine industry, both in terms of loss of athletic performance and amount of money spent for diagnostics and treatments (Ross et al. 1998).

A survey in a mixed horse population reported that common risk factors for lameness include athletic activity, housing in small operations, and the male or male-castrated gender (Ross et al. 1998). For racehorses, a large amount of information is collected in data bases and multivariate techniques enable the investigation of individual risk factors for musculoskeletal injury and lameness, thereby allowing a more sophisticated approach to study the complex interaction between many variables. Risk factors identified for musculoskeletal injury in Thoroughbreds include age, sex, shoe type, track type and surface, track condition, season, intensity of racing and training, days since the last race, and type of racing activity (flat racing, hurdle or steeple chase) performed (Bailey et al. 1998; Estberg et al. 1996; Hernandez et al. 2001; Kane et al. 1996; Mohammed et al. 1991). The properties of facilities for training and competition also appear to have a major influence on the occurrence of musculoskeletal injuries in
other performance horses. A study in racing Standardbreds showed that increasing the banking of the turns of the racetrack caused a significantly lower incidence of musculoskeletal injuries and lameness (Evans and Walsh 1997).

Wastage is a term used to describe both the phenomenon of lost performance in horses from conception to adulthood due to death or injuries (i.e. they never perform), or days lost due to inability to train or compete (Olivier et al. 1997). Multiple surveys in populations of Thoroughbred racehorses (Jeffcott et al. 1982; Kobluk et al. 1990; Lindner and Dingerkus 1993; Olivier et al. 1997; Rossdale et al. 1985; von Herzog and Lindner 1992) and Standardbreds (Physick-Sheard 1986a; Physick-Sheard 1986b; Physick-Sheard and Russell 1986) consistently identified lameness as the most prevalent cause for wastage. With 72.1% and 67.6%, respectively, the percentage of training days lost attributable to disorders causing lameness was comparable between Thoroughbred racehorses in South Africa (Olivier et al. 1997) and England (Jeffcott et al. 1982). In a survey of Thoroughbred racehorses in Germany, musculoskeletal problems were responsible for 55.3% of the horses retiring from racing (von Herzog and Lindner 1992). A study in a different German racing Thoroughbred population attributed 57% of the wastage occurring due to lameness (Lindner and Dingerkus 1993). A survey within a Thoroughbred racehorse population in the United States reported an estimated 44% of horses having a musculoskeletal problem preventing them from training or racing anytime during the survey period, and an additional 32% of horses having a musculoskeletal problem requiring alterations in their training and racing schedules (Kobluk et al. 1990). During a single racing season in England, Jeffcott et al. (1982) observed the majority of causes for lameness in a population of 164 racehorses in the
forelimb (70%), with lesions in the foot (15.4%), dorsal metacarpus (15.4%), fetlock (15.0%), and carpus (12.6%) being the most prevalent sites of lameness. In 21 cases (8.5%), the site of lameness could not be determined (Jeffcott et al. 1982). These findings are in agreement with another survey from England by Rossdale et al. (1985) who detected a comparable distribution of the sites of lameness in 198 racing Thoroughbreds over two seasons (Rossdale et al. 1985). However, the percentage of cases where the site of the lameness could not be localized (31.2%) was considerably greater than in Jeffcott’s survey.

In daily practice, the origin or the site of lameness in a horse is often suspected by a process of elimination of other (common) sites, but the exact origin may actually remain unknown (Rossdale et al. 1985). Many conditions that cause lameness respond favorably to restricted exercise and/or administration of non-steroidal anti-inflammatory drugs (NSAIDs), and precise localization of the cause of lameness is not attempted due to the time and costs associated with a thorough lameness examination including intrasynovial and peripheral nerve blocks, and diagnostic imaging (Rossdale et al. 1985).

Lameness is an important economical factor in performance horses, not only due to costs arising from diagnostics and treatments, but also due to the loss of generated income (money won). Lameness disorders therefore represent a challenge to the equine practitioner who wants to provide pain relief, reinstitute athletic use of the horse, and minimize economic loss, while at the same time operate within ethical and regulatory constraints of modern competition. This generates a growing interest in economical, alternative treatment modalities that appear to result in an improved and accelerated
healing process and shorter convalescence periods, such as extracorporeal shock wave therapy (ESWT).

2.2 Extracorporeal Shock Waves

2.2.1 Characteristics of Extracorporeal Shock Waves

Extracorporeal shock waves (ESW) are acoustic pressure gradient waves that are created by a generator and propagated into the patient’s body where they exert their effects on tissues. They are characterized by positive pressures of up to 100 megapascals (MPa), followed by a rapid fall to negative pressures of 5-10 MPa. One MPa corresponds to approximately 10 times the atmospheric pressure. Extracorporeal shock waves build up very rapidly with a rise time of 30-120 nsec, and their pulse duration is approximately 300 to 500 nsec (Sturtevant 1996). Shock waves are differentiated from other acoustic waves (e.g. ultrasound waves) by their lower frequencies, their lower tissue absorption, and the absence of thermal effects within tissues. Therapeutic ESW travel nearly unchanged through fluids and soft tissues of the body and exert their effects at sites where there is a change in acoustic impedance along their path. Energy is released at these interfaces of different impedance values, and this creates compression and shear loads on the surface of the material with greater impedance. Rapid interaction between compression and shear forces results in a process referred to as cavitation. Microscopic gas bubbles are built up on the surface of the material with greater impedance and the collapse of these bubbles creates a small jet of liquid (fast flow) that causes high local stresses. Cavitation effects are believed to be responsible for the effects of ESW in tissues (Sturtevant 1996).
Although the exact mechanism of how ESW exert their effects in tissues is poorly understood, four consecutive reaction phases are postulated to occur in the body (Haupt 1997). In the physical phase, extracellular cavitations, ionization of molecules and an increase in cell membrane permeability occur as a direct effect of ESW. The subsequent physical-chemical phase consists of an interaction between diffusible radicals and biomolecules released from ESW-stimulated cells. This results in the chemical phase characterized by intracellular reactions and molecular changes in the cells. The biological phase is established if the changes occurring in the chemical phase persist (Haupt 1997).

Two fundamentally different types of shock waves (focused and non-focused shock waves or radial pressure waves) are used for extracorporeal shock wave therapy (ESWT) in humans and animals (McClure and Merritt 2003). Following their generation in a fluid medium, focused shock waves are concentrated (focused) and directed by reflection at target zones in the patient’s body (Sturtevant 1996). Focused ESW are created and focused from a wide arc so that at the site of actual wave generation, the amount of energy dispersed to the periphery is minimal (Sturtevant 1996). Via a focusing mechanism, each shock wave is directed toward a clearly defined point within the body of the patient; thus the wave energy concentrated at this point is extremely high. This point is referred to as the focal point and its location within the patient can be changed by adjusting the energy output (voltage) of the generator or by adjusting the focusing mechanism (Sturtevant 1996). In stationary focused ESW generators, diagnostic imaging techniques, such as fluoroscopy, radiography or ultrasound, are used to exactly localize the focal point (Schnewlin and Lischer 2001). This minimizes surrounding soft tissue and organ damage because the maximal (therapeutic) wave energy is reached only at the focal...
point (Schnewlin and Lischer 2001; Sturtevant 1996). The energy that focused ESW exert at the focal point is expressed as *energy flux density* and is recorded in energy per surface area units (mJ/mm²). The more focused the emitted shock waves, the greater is the energy flux density achieved at the focal point (Sturtevant 1996).

Focused shock waves are generated by initiating a pressure wave within a fluid medium. Three different mechanisms are currently used to generate focused shock waves. *Piezoelectric systems* utilize a crystalline material that, when stimulated with high voltage electricity can expand or contract and initiate a pressure wave in surrounding fluid. *Electromagnetic systems* have coils that create opposing magnetic fields when an electric current is applied to them, causing a submerged membrane to move and generate a pressure wave in the surrounding fluid medium. *Electrohydraulic systems* create a plasma bubble via generation of a high voltage spark. Expansion of the plasma bubble compresses the surrounding fluid medium, thereby creating a pressure wave. Although all three mechanisms have different waveforms and energy characteristics, they all generate ESW that appear to be sufficiently intense to exert tissue effects at the focal point (Sturtevant 1996). Recently, portable electrohydraulic and piezoelectric systems have been introduced into the veterinary market to treat musculoskeletal disorders in horses. In these units, focused ESW are generated and reflected in handheld probes that are applied to the limb or body of the horse.

*Non-focused extracorporeal shock waves* or *radial pressure waves* are created by mechanical concussion and appear as expanding wave fronts that rapidly attenuate after their generation (Ammendolia et al. 2001; McClure and Merritt 2003). Compared to focused ESW, they are characterized by lower wave energies, a slower rise time, and a
negative component that is of the same magnitude as the preceding positive component (McClure and Merritt 2003). Non-focused ESW are not reflected or redirected toward a focal point in the patient’s body and their wave energy declines rapidly in proportion to the distance from the site of their generation. Non-focused ESW are transmitted up to 35 mm into the patient’s body (Ammendolia et al. 2001). They are generated by transforming kinetic energy from an accelerated piston within a handheld applicator to mechanical force. The applicator is applied to the patient’s skin surface for treatment. Since non-focused ESW differ considerably from focused ESW, the term “shock wave” may be inappropriate to describe them (Schnewlin and Lischer 2001). However, beneficial effects in treatment of various orthopedic disorders in man (Gremion et al. 2001; Lohrer et al. 2001a; Lohrer et al. 2001b) and horses (Boening et al. 2000; Palmer 2002) with non-focused ESW have been reported. Due to their affordability and their small size, portable units for non-focused ESWT have gained substantial popularity in equine practice.

2.2.2 Lithotripsy

Lithotripsy was the first reported medical application of ESW. The literal meaning of the term lithotripsy is “the act of breaking stones” (Adams and Senior 1999). Initially, mechanical devices were developed in human medicine to crush or fragment urinary concretions (uroliths) in the urinary bladder. These devices reduced uroliths to multiple fragments that could be flushed through a cystoscope sheath or pass the urethra without causing further obstruction (Adams and Senior 1999). The major drawback of these mechanical lithotripsy systems was their size, which precluded their use in more confined locations such as the urethra, ureter and renal pelvis. Their use in smaller
patients such as children, cats and small dogs was impractical, and these patients instead had to undergo open surgery procedures for the removal of urinary calculi (Senior 1984).

In the early 1970’s, Haeusler and Kiefer demonstrated that shock waves are capable of disintegrating human renal stones in vitro (Haeusler and Kiefer 1971). Subsequently, electrohydraulic shock-wave lithotripsy (EHL) was developed to fragment uroliths in people without the need for large crushing devices (Adams and Senior 1999). The EHL electrode generates a spark in the fluid surrounding the concretion. Generation and subsequent disappearance of this spark results in large pulse pressures (shock waves), which are absorbed by the concretion and lead to its disintegration (Watson 1970). Because the electrode wire used for EHL is quite small, this technique allows lithotripsy in less accessible anatomical locations, such as the urethra, ureter and renal pelvis (Adams and Senior 1999; Mitchell and Kerr 1977; Raney 1975). Electrohydraulic lithotripsy has also been used in veterinary medicine to disintegrate cystic calculi in dogs (Senior 1984), and cystic (Eustace and Hunt 1988; MacHarg et al. 1985) and ureteral (Rodger et al. 1995) calculi in horses.

The development of extracorporeal shock wave lithotripsy (ESWL) in the early 1980’s was considered a major breakthrough in the treatment of uroliths, because it allows disintegration of stones in the upper urinary tract non-invasively from outside the body (Chaussy et al. 1980; Chaussy et al. 1982; Lingeman et al. 1987). In ESWL, shock waves are generated in the fluid medium of a wave generator and then transmitted percutaneously through the soft tissues of the patient to the urolith. Wave transmission into the body can be accomplished by submerging the patient in a water bath, or by using a fluid- or gel-filled cushion that is placed against the patient’s skin over the target area.
The latter is referred to as second or third generation or “dry” lithotripsy (Auge and Preminger 2002). Repeatedly administered shock waves cause disintegration of the urolith into small fragments that can be passed through the distal urinary tract. Currently, ESWL is considered to be the treatment of choice for 70 to 80% of people with uroliths (Chaussy and Fuchs 1989; Lingeman et al. 1987). An additional 25% of patients with more complex stones in the upper urinary tract can be treated with lithotripsy in combination with endourological procedures and do not have to undergo invasive open surgery (Chaussy and Fuchs 1989).

Extracorporeal shock wave lithotripsy has also been used to treat nephroliths and ureteroliths in dogs (Adams and Senior 1999; Block et al. 1996). In animals, ESWL must be performed under general anesthesia to allow for adequate positioning and analgesia. Approximately 30% of dogs treated with ESWL require two treatments to disintegrate uroliths into fragments that are sufficiently small to traverse the ureter (Adams and Senior 1999). These success rates are comparable with those of ESWL of nephroliths in people where first treatment success rates of 76% to 99% have been reported (Psihamis et al. 1992; Wilson and Preminger 1990).

Although ESWL of nephroliths was at first not believed to cause damage to the kidney (Chaussy and Schmidt 1984), studies in people, dogs, and minipigs showed that it causes intrarenal hemorrhage (Hill et al. 1990; Koga et al. 1996; Lingeman et al. 1989; Newman et al. 1987). The severity of damage caused by ESW depends on dose (voltage and number of shock waves) and frequency (number of shock waves per second) of the treatment (Lingeman et al. 1989; Newman et al. 1987). A study revealed that renal damage occurs mainly in the tubules and peritubular capillaries, and that the glomerular
filtration rate and renal blood flow return to pre-treatment levels by one week after ESWL (Hill et al. 1990).

After the successful introduction of ESWL for destruction of uroliths, concretions in other organs of human patients such as gallbladder, bile duct, pancreas and salivary glands have also been successfully treated with ESWL (Iro et al. 1989; Iro et al. 1992; Sauerbruch et al. 1986; Sauerbruch et al. 1987).

2.2.3 Important Animal Models

Prior to the clinical application of ESW for the treatment of musculoskeletal disorders their effects on tissues were extensively investigated in animal models. Initial studies in piglets revealed a dose-dependent stimulation of wound healing in irradiated and non-irradiated split-thickness skin wounds (Haupt and Chvapil 1990). These findings led to the hypothesis that fracture healing might also be enhanced by shock waves.

Delius et al. (1995) observed periosteal detachment and extensive subperiosteal hemorrhage in specimens collected from rabbits immediately after a single application of focused shock waves to the stifle. Intense apposition of new lamellar bone and considerable cortical thickening of the bone were observed in samples that were obtained several weeks after treatment (Delius et al. 1995). Comparable observations were made in a dog model by Ikeda et al. (1999) after shock wave application to the femur (Ikeda et al. 1999). Radiographic, histological and biochemical findings in a rat model suggested that fracture healing was stimulated by ESW (Haupt et al. 1990). These findings were corroborated in a canine model (Johannes et al. 1994), where all ESW-treated dogs demonstrated a radiographically evident bony union of an osteotomized radius at twelve weeks after a single treatment with focused ESW, compared with only one bony union
that was observed in the untreated control group (Johannes et al. 1994). In a sheep osteotomy model, treatment with focused ESW resulted in a smaller fracture gap and enhanced periosteal and endosteal bone apposition on radiographs (Ekkernkamp et al. 1991). The radiographic findings in that study were confirmed by histological and fluorescence microscopic analysis where accelerated apposition of stable lamellar bone was observed (Ekkernkamp et al. 1991). In a rabbit osteotomy model, ESW application resulted in such exuberant callus formation that the overall process of fracture healing and bone remodeling was actually delayed (McCormack et al. 1996).

Shock wave-induced bone formation and remodeling is believed to be caused by microfractures within the bone which act as local stimulators of bone remodeling (Kaulesar Sukul et al. 1993; Valchanou and Michailov 1991). In a dog femoral osteotomy model, hemorrhage, detachment of the periosteum, and small fractures on the inner surface of the cortex were observed immediately after application of 500 or more focused ESW (Ikeda et al. 1999). Initial studies conducted in horses, however, failed to identify the presence of microfractures after treatment with focused ESW (McClure et al. 2000).

In an attempt to investigate the effect of ESW on equine bone, McClure et al. (2000) applied 1,000 pulses of focused ESW dorsally to metacarpal and metatarsal bones of horses under general anesthesia. All animals were euthanatized immediately after treatment and treated and non-treated bones were evaluated macroscopically and microscopically. In agreement with studies in smaller species (Delius et al. 1995; Ikeda et al. 1999), ESW induced subperiosteal and endosteal hemorrhage at the application sites. However, no microfractures and no other lesions in skin, tendons, suspensory ligaments
and associated neurovascular structures of treated limbs were observed (McClure et al. 2000). A recent histomorphometrical ex-vivo study in third metacarpal and metatarsal bone segments from horses revealed small but significant effects of ESW on the appearance of microcracks in cortical bone (Da Costa Gomez et al. 2004). Whereas application of focused ESW resulted in an increased density of bone microcracks compared with untreated bone segments, an increase in microcrack length was observed after non-focused ESW application (Da Costa Gomez et al. 2004).

Because of the proximity of many ESWT application sites to synovial joints, possible side effects of ESW on articular cartilage were investigated in a rabbit model by Vaeterlein et al. (2000). No significant radiographic, gross or histological pathological changes were observed in physeal and articular cartilage after ESW application to the femoral condyle in growing rabbits (Vaeterlein et al. 2000).

2.2.4 Orthopedic Applications in Humans

2.2.4.1 Pseudarthrosis

In people, 0.5 to 10% of long bone fractures may exhibit delayed or insufficient healing and may ultimately result in pseudarthrosis (Bischof and Kinzl 2000). Success rates for treatment of pseudarthrosis with ESWT have been reported to vary between 21% and 97% (Haist 1995; Haupt 1997; Ikeda et al. 1999; Schleeberger and Senge 1992). In one study, the patients were differentiated with regard to hypertrophic versus atrophic forms of pseudarthrosis based on radiographic criteria. While the majority of patients with hypertrophic pseudarthrosis revealed complete healing, healing after ESWT was rarely observed in patients with atrophic pseudarthrosis (Haist 1995). Complications following ESWT for pseudarthroses include transient local pain, petechial subcutaneous
bleeding and hematomas. These were observed in up to 4% of human patients (Haupt 1997).

### 2.2.4.2 Hip Endoprosthesis

Implant instability has been identified as the most prevalent cause of failure in hip endoprosthesis (Ahnfeldt et al. 1990). This condition is usually addressed by replacement of the loose prosthesis. Extracorporeal shock waves have been investigated for their potential use to loosen the bone cement surrounding the implant in order to facilitate removal of the old prosthesis prior to replacement (Karpman et al. 1987; May et al. 1990; Weinstein et al. 1988). However, these attempts were unsuccessful and the failure was explained by the similar acoustic properties of bone and bone cement (Delius 1995; Weinstein et al. 1988).

### 2.2.4.3 Osteochondrosis

One investigator treated people with osteochondrosis dissecans of the stifle and talus with ESWT and observed re-attachment of fragments to the parent bone in several cases (Schleeberger 1995). Other ESW applications with promising results have also been performed in patients with Koehler’s Disease, Perthes’ Disease or Osgood-Schlatter’s Disease (Haist and von Keitz-Steeger 1995; Schleeberger 1995). To date, however, it appears that the use of ESW for the treatment of osteochondrosis has to be considered experimental (Haupt 1997).

### 2.2.4.4 Calcific Tendonitis

Calcium deposits in the soft tissues of the shoulder represent a common and often incidental radiographic finding in people. Deposits develop most often in the supraspinatus tendon and can be detected in 2 to 20% of people without symptoms of
shoulder pain (Bosworth 1941). However, significantly more patients with shoulder pain (up to 50%) have calcium deposits (Bosworth 1941). Spontaneous resolution of the calcium deposits and associated clinical signs has been observed in up to 100% of people with acute shoulder pain (Harmon 1958). However, chronic recurrent shoulder pain is often associated with persistent discomfort and calcium deposits on radiographs. Conventional treatment for calcific tendonitis includes physical therapy, NSAIDs, radiotherapy, steroid infiltration, needling (breaking up calcium deposits with hypodermic needles), and arthroscopic or open surgical procedures (Haupt 1997). In one study, twenty patients with severe shoulder pain and calcium deposits of at least 1 cm diameter were treated with focused ESW. Twelve weeks later, seven patients showed complete radiographic disappearance of the deposits, whereas partial disintegration was observed in five patients. Six patients were completely pain-free, eight were substantially improved and only one patient experienced clinical deterioration (Loew et al. 1995). Two other case series of patients with calcific tendonitis reported complete resolution of shoulder pain or substantial clinical improvement in 73% and 83% of patients treated with ESWT, respectively (Haupt and Katzmeier 1995; Rompe et al. 1995). The specific mechanism of action of ESW on the calcium deposits is unknown. One investigator postulated that resorption of the calcium deposits within the tendon occurs and that this process is preceded by mechanical breakdown and by reactive local neovascularization (Loew et al. 1995). The potential of ESW to induce neovascularization has recently been demonstrated in a canine model where new capillary-formation was observed at the tendon-bone interface of Achilles tendon specimens after application of low energy ESW (Wang et al. 2002).
2.2.4.5 Soft Tissue Pain Syndromes

Painful tendinopathies in people, such as tennis elbow (epicondylitis humeri radialis), golfer’s elbow (epicondylitis humeri ulnaris), or shoulder pain (periarthritis humero-scapularis) have historically been treated with combinations of immobilization, physical therapy, radiotherapy and local steroid injections with variable success rates (Haupt 1997). Surgical treatments, such as the release of the common forearm extensor origin in severe cases of tennis elbow, are usually restricted to patients that are unresponsive to non-surgical treatment, but these procedures appear to yield good to excellent results (Haupt 1997; Verhaar 1994).

Dahmen et al. (1992) were the first to use ESWT to treat patients with soft tissue pain in proximity to bones. A variety of more than 30 different syndromes were treated in 512 patients for whom surgery was originally intended. Fifty-two percent of the cases experienced excellent results, 28% showed substantial improvement and only 3% ultimately required surgical procedures (Dahmen et al. 1992). Haist and von Keitz-Steeger (1995, 1996) reported success rates of 86% and 74% for treatment of tennis elbow and shoulder pain with focused ESW, respectively (Haist and von Keitz-Steeger 1995; Haist and von Keitz-Steeger 1996). In another investigation, focused ESW were used to treat 150 people with tennis elbow that had undergone unsuccessful conservative treatment attempts for at least the previous three months (Rompe et al. 1995). A success rate of 84% in a population of patients that would otherwise have required surgical treatment was observed (Rompe et al. 1995). Lohrer et al. (2001) treated 55 patients with tennis elbow with up to three applications of non-focused ESW. Inclusion in this study required at least two unsuccessful conservative treatment attempts during the six months...
previously and a clinical indication for surgical treatment. Fifty-six percent of the patients that were previously limited in their activities revealed resolution of all symptoms after 12 months (Lohrer et al. 2001a).

The exact mechanism how ESW exert their reversible analgesic effect in soft tissue pain syndromes still needs to be elucidated. Clinically, a biphasic analgesic response is observed in people (Ogden and Ogden 2002). An immediate relief appears to be followed by a return of the pain at three to four days after treatment with ESW and then a second gradual improvement over the ensuing 3 to 4 weeks occurs (Ogden and Ogden 2002).

Haist and von Keitz-Steeger (1995) proposed three hypotheses for how ESW could induce local analgesia: 1) ESW induce cell membrane damage and non-functional nociceptors are subsequently unable to build up a membrane potential to transmit pain signals; 2) the nociceptors are over-stimulated by ESW and emit high frequency impulses that are modulated by a gate control mechanism; and 3) ESW-induced free radicals change the cellular environment and induce the release of unknown local pain-suppressing substances (Haist and von Keitz-Steeger 1995). The gate control hypothesis is somewhat corroborated by the results of an ex-vivo study where electromyographic measurements from an isolated frog sciatic nerve in an organ bath revealed that approximately 95% of shock waves applied to the nerve were able to induce action potentials that were lower in amplitude but similar in shape to compound action potentials evoked by direct electrical stimulation (Schelling et al. 1994). With increasing dose and frequency of application, ESW are capable of inducing considerable morphological damage in peripheral nervous tissues. In one study, marked structural
abnormalities were observed in peripheral nerves of mice after application of high-energy ESW to the calf muscles (Smits et al. 1993).

2.2.5. Orthopedic Applications in Veterinary Medicine

In the late 1990’s, ESW were introduced into veterinary medicine to treat musculoskeletal disorders in horses (McCarrol 1999). To date, however, no controlled studies for the use of this new treatment modality in horses have been published. Various case series reporting encouraging results for the treatment of orthopedic disorders in horses have been presented at scientific meetings (Boening et al. 2000; Crowe et al. 2002; McCarrol 1999; Palmer 2002; Scheuch et al. 2000).

Horses are usually treated with two to three treatments of medium to high-energy ESW administered at three-week intervals (Schnewlin and Lischer 2001). The hair over the target area is clipped and the shock waves are transmitted into the horse’s body through a fluid- or gel-filled cushion (focused ESW) or by placing the applicator with the piston against the horse’s skin (non-focused ESW). Use of a coupling gel is usually necessary to optimize shock wave transmission and to minimize energy loss.

Treatment with both non-focused and focused ESW can be associated with considerable irritation and may be resented by the animals. Sedatives and manual restraint (e.g. a nose twitch) are often necessary to administer the ESWT regimen in standing horses (Boening et al. 2000; Palmer 2002). Occasionally, peripheral nerve blocks are placed prior to ESWT application, or treatment is performed under general anesthesia (McCarrol and McClure 2000).

Non-focusing shock wave generators initially gained substantial popularity in equine practice due to their small size and their affordability. The comparison between
non-focused (radial) and focused ESW generators and their therapeutic effects appears to be somewhat difficult due to the different characteristics of the shock waves they generate. Recently, portable electrohydraulic and piezoelectric systems have been introduced into the veterinary market to treat musculoskeletal disorders in horses.

2.2.5.1  Suspensory Desmitis and Flexor Tendon Injuries

Lesions at the origin of the suspensory ligament account for approximately 5% of forelimb lameness in performance horses (Dyson 1992). Pain at the suspensory origin can be caused by suspensory ligament desmitis, torn Sharpey’s fibers at the insertion of the ligament, or even avulsion fractures of the origin (Bertone 2002). Flexor tendonitis, particularly tendonitis of the superficial digital flexor (SDF) tendon, is a common cause of lameness in racehorses and other performance horses (McIlwraith 2002). Lesions in these structures range from minor tearing of fibers to complete rupture of the tendon. Similar to suspensory desmitis, flexor tendonitis results in a high morbidity with a prolonged recovery period (McIlwraith 2002).

In one study, 30 horses with chronic suspensory ligament desmitis were treated with three applications of non-focused ESWT at two- to four-week intervals. Four weeks after the last treatment, 16 horses were free of lameness and 9 horses had improved at least one lameness grade (Boening et al. 2000). Donati et al. (2001) treated a total of 202 performance horses with acute and chronic flexor tendon injuries or supensory ligament desmitis with focused ESWT under ultrasonographic control. Up to three treatments were administered to the horses standing under chemical restraint. After an average follow-up of 30 months, a complete resolution of clinical signs was observed in 87% of horses (Donati et al. 2001).
2.2.5.2  **Dorsal Metacarpal Disease**

Periostitis and stress fractures of the dorsal surface of the third metacarpal bone constitute a spectrum of diseases that are commonly observed in young fast-gaited horses of two to three years of age (Bertone 2002). Although the greatest incidence is observed in Thoroughbred racehorses, it can also affect young Quarter Horses and occasionally racing Standardbreds. This disease complex is referred to as dorsal metacarpal disease (DMD) and is believed to occur due to a failure of the adaptive response of the third metacarpal bone to altered biomechanical stresses arising from high-speed training and racing (Bertone 2002).

Palmer (2002) treated 50 Thoroughbred racehorses with DMD with non-focused ESWT. All horses meeting clinical and radiographic inclusion criteria for the study had been treated unsuccessfully for at least two months previously with combinations of stall rest, restricted exercise, percutaneous periosteal scraping, osteostixis, and NSAIDs. After three treatments at three-week intervals, 40 horses (80%) resumed speed-work consisting of breezing and racing without lameness or other clinical signs associated with DMD (Palmer 2002). In another study, 10 horses with dorsal or palmar metacarpal cortical stress fractures were treated one to three times with focused ESW applied under ultrasonographic control. Ninety days after the last treatment, eight horses (80%) had returned to racing or training and six (60%) were pain-free and/or revealed radiographic signs of fracture healing (Scheuch et al. 2000).

2.2.5.3  **Degenerative Joint Disease**

Degenerative joint disease (DJD) is the common end-stage of a large variety of joint disorders and is characterized by progressive deterioration of articular cartilage
accompanied by changes in the subchondral bone and the soft tissue components of the joint (McIlwraith 2002). Pain and functional restriction associated with DJD account for a majority of clinically observed lameness disorders in horses.

McCarrol and McClure (2000) treated 75 horses with DJD of the distal intertarsal and tarsometatarsal joints (bone spavin) with one treatment of 2,000 pulses of electrohydraulic focused ESW. All horses were treated under general anesthesia and under fluoroscopic control. After ninety days, 59 horses (80%) had improved at least one lameness grade and 13 (18%) were completely sound. In all horses, no significant changes in radiographic findings (e.g. ankylosis of the distal tarsal joints) were observed compared with radiographs obtained prior to treatment. The investigators believed that clinical improvement in these horses resulted either from an ESW-induced analgesic effect or from altered shock absorption properties of the distal tarsal bones due to adaptive remodeling after treatment (McCarrol and McClure 2000).

2.2.5.4  Navicular Disease

Navicular disease represents one of the most common and controversial causes of intermittent forelimb lameness in horses between four and 15 years of age (Ackerman et al. 1977; Stashak 2002b). It has been estimated that navicular disease accounts for one-third of all cases of chronic forelimb lameness in horses (Colles 1982).

Non-surgical treatment for navicular disease includes various types of corrective shoeing in combination with NSAIDs, medication with hemorheologic agents such as isoxsuprine hydrochloride, pentoxifylline and propentofylline, injection of corticosteroids and/or sodium hyaluronate in the distal interphalangeal joints or in the podotrochlear bursa, intramuscular injection of polysulfated glycosaminoglycans, and oral
supplementation of glucosamine hydrochloride and sodium chondroitin sulfate (Stashak 2002b). Surgical treatment is usually reserved for cases that are unresponsive to non-surgical treatment. Currently, palmar digital neurectomy represents the most commonly performed surgical treatment for navicular syndrome. This procedure desensitizes the caudal one-third to one-half of the palmar foot region and the sole extending dorsally to the toe (Stashak 2002b).

Garcia Lineiro and Echezarreta (2001) treated 15 horses with navicular disease with five treatments of 3,000 pulses of non-focused ESW at one week-intervals. All horses included in the study had clinical signs for over six months, presented with at least a grade 3/5 lameness, and had previously undergone unsuccessful medical and/or surgical treatment attempts. The ESW treatment was applied percutaneously over the palmar digital nerves and over the palmar surface of the navicular bone. In all animals, improvement of the lameness was observed after the third treatment and the lameness had completely resolved in all horses after the fifth treatment. All horses in the study remained sound for of at least 60 days (Garcia Lineiro and Echezarreta 2001). Another study reported on the treatment of 26 horses with one application of electrohydraulic focused ESW under general anesthesia. Complete resolution of lameness was observed in all horses after 6 months (Baer et al. 2001). In another investigation where lower doses of focused ESW were utilized, a substantially lower success rate was reported for the treatment of navicular disease (Scheuch et al. 2000).
2.3 Morphology and Function of Peripheral Nerves

2.3.1 Anatomy and Physiology

The axons of peripheral nerves are surrounded by three different layers of connective tissue (Kimura 1989a). The endoneurium provides the supporting structure around individual axons within the nerve fascicles. The perineurium consists of collagenous tissue that binds each fascicle with elastic fibers and mesothelial cells. This layer also provides a diffusion barrier to regulate intrafascicular fluid (Ross and Reith 1969). The epineurium is composed of collagen tissue, elastic fibers and fatty tissue and binds individual fascicles together (Kimura 1989a).

Peripheral nerves contain myelinated and unmyelinated fibers. Multiple inherent factors determine whether or not myelination of an axon will occur (Kimura 1989a). In myelinated fibers, the surface membrane of a Schwann cell (axolemma) spirals around the axon to form the myelin sheath. Each myelinated axon has its own Schwann cells, which regulate the myelin volume and thereby the thickness of the myelin sheath (Smith et al. 1982). The nodes of Ranvier, located at junctions between adjacent Schwann cells along the axon, represent gaps that are not insulated by Schwann cell processes. In myelinated nerve fibers, action potentials propagate from one node of Ranvier to another and the rate of propagation is approximately proportional to the fiber diameter. The spacing of the Schwann cells at the time of myelination determines the internodal distance. As the nerve grows in length during development, the internodal distance must increase because Schwann cells are unable to proliferate. Thus, the fibers myelinated early achieve larger diameters and wider spacing of the nodes of Ranvier (Vizoso and Young 1948).
In contrast to myelinated axons, several unmyelinated fibers share Schwann cells that give rise to multiple separate processes, each surrounding one axon (Gamble and Eames 1964). In unmyelinated fibers, conduction velocity varies in proportion to the square root of fiber diameter (Kimura 1989a).

Axons of nerve cells have electrical properties common to all excitable cells in the body. The inside of the nerve fiber is negatively charged with a transmembrane steady-state potential that measures from -20 to -100 mV depending on the exact location (Kimura 1989a). The somatic membrane of the nerve cell body has less polarization (-70 mV) than the axon (-90 mV). Application of a weak current to a nerve causes negative charges to accumulate outside the axon membrane, making the inside of the cell relatively more positive (cathodal depolarization). Under the positive pole (anode), the negative charges tend to leave the membrane surface, making the inside of the cell relatively more negative (anodal hyperpolarization). After about 10 to 30 mV of depolarization, the membrane reaches the critical level for initiation of an action potential with a consistent response, regardless of the kind of stimulus or its magnitude. An action potential initiated along the course of an axon propagates in both directions from its point of origin. Intracellular current flows from the active area to the adjacent negatively charged inactive area. An opposing current flows through the extracellular fluid from the inactive to the active region of the axon. This local current depolarizes inactive regions on both sides of the active area. Once it reaches the critical level, an action potential generates a new local current further distally or proximally along the axon (Kimura 1989a).
In myelinated fibers, action potentials can only occur at the nodes of Ranvier. This results in a local current jumping from one node to the next (saltatory conduction) instead of a continuous propagation as it is observed in unmyelinated fibers (Kimura 1989a).

Various factors affect the time necessary for the generation of action potentials, which in turn determines the conduction velocity of an axon. Rapid propagation and fast conduction velocity result from faster rates of action potential generation, increased current flow along the axons, lower depolarization thresholds of the axonal membrane, and higher temperature which facilitates depolarization by increasing sodium conductance toward the intracellular fluid (Kimura 1989a). The longitudinal resistance of the axoplasm tends to inhibit the flow of local current. In myelinated axons, the capacitance and conductance of the internodal membrane have the same effect resulting in loss of current before it reaches the next node of Ranvier. This increases the time required to depolarize the adjacent nodal membrane, thereby decreasing conduction velocity. Both internodal capacitance and conductance decrease with myelin thickness. For a fixed axonal diameter, conduction velocity increases with myelin thickness only up to a certain point. An increase in myelin thickness has two opposing effects on conduction velocity; a larger axoplasmic resistance on one hand, and smaller membrane conductance and capacitance on the other (Waxman 1980). Damaged nerve fibers with demyelinated or partially remyelinated nerve fibers have an increased internodal capacitance and conductance because of their thin myelin sheath. More local current is lost to charge the capacitance and by leakage through the internodal membrane before reaching the next node of Ranvier (Kimura 1989a). Thus, demyelinated axons
characteristically have conduction failure, decreased conduction velocities, and temporal dispersion (Waxman 1980).

Conduction abnormalities in myelinated axons do not necessarily imply the presence of demyelination, but can also result from compression. The decreased fiber diameter in this case should actually facilitate conduction. Concomitant increase in resistance in the axoplasm, however, more than offsets this effect by delaying the flow of current to the next node of Ranvier (Kimura 1989a).

Peripheral nerves contain different groups of nerve fibers with different conduction velocities. Each class of nerve fibers is represented by a characteristic peak in the compound action potential (CAP) elicited by supramaximal electric stimulation (Kimura 1989b). A-fibers represent afferent or efferent myelinated somatic axons. In sensory nerves, they are subdivided in four groups (I to IV), whereas in efferent muscle nerves they are subdivided into alpha and gamma motoneurons, according to their fiber diameter. Cutaneous somatic nerves contain myelinated alpha and delta A-fibers and are also subdivided according to their diameter. The efferent unmyelinated C-fibers consist of the efferent postganglionic axons of autonomic nerves and the small afferent axons of the dorsal root and peripheral nerves. Several C-fibers share a single Schwann cell, unlike A- and B-fibers which are individually insulated. This and the absence of the myelin sheath allow histological identification of the C-fibers (Kimura 1989a).

2.3.2 Classification of Nerve Injuries

Three degrees of peripheral nerve injuries have been described (Seddon 1943). *Neuropraxia* represents bruising or conduction loss without actual structural damage to the axon. Complete recovery usually takes place within days or weeks after removal of
the cause. The conduction velocity of the nerve, if initially slowed because of associated demyelination, returns to normal with repair of the myelin sheath. Mechanical factors have been identified as the major factor for the development of neuropraxia (Kimura 1989b); however, short term changes in nerve conduction can also be induced by anoxia secondary to ischemia (Lewis et al. 1931). In axonotmesis, axonal damage results in the loss of continuity and Wallerian degeneration of the distal segment of the nerve fiber (Seddon 1943). Conduction block occurs immediately at the site of injury, followed by an irreversible loss of excitability, first at the neuromuscular junction, then at the distal nerve segment. The time course of Wallerian degeneration varies considerably among different species, but it does generally not occur until 4 or 5 days after acute interruption of nerve continuity (Gilliatt and Taylor 1959). Functional recovery depends on regeneration of nerve fibers and can take months or years due to their slow growth rate (1 to 3 mm per day). Neurotmesis is referred to as an injury that separates the entire nerve and disrupts supporting connective tissue structures. Functional recovery after neurotmesis depends on whether the perineurium and the architecture of the nerve sheath were severed at the time of injury (Kimura 1989a).

### 2.3.3 Principles of Nerve Conduction Studies

Measurement of conduction characteristics represents a simple and reliable testing method of peripheral nerve function. With adequate standardization, this method allows not only identification of a lesion in a nerve, but also precise localization of the site of maximal involvement. Electrical stimulation of a nerve is used to initiate an impulse that travels along motor, sensory, or mixed nerve fibers. Assessment of conduction characteristics depends on the analysis of evoked compound action potentials (CAP) that
are recorded either on the muscle if evaluating motor fibers or from the nerve itself if
evaluating sensory nerve fibers (Kimura 1989b). Electrical stimulation is used in most
clinical studies; however, tactile stimulation has also been shown to elicit recordable
nerve action potentials (Buchthal 1965). In an ex-vivo study using frog sciatic nerves in
organ baths, approximately 95% of shock waves that were applied to the nerve were able
to induce action potentials that were lower in amplitude but similar in shape to the
compound action potentials evoked by electrical stimulation in conduction studies
(Schelling et al. 1994).

Surface electrodes or needle electrodes can be used for electrical stimulation of a
nerve. Needle electrodes placed subcutaneously close to the nerve require much less
current to elicit the same response in the nerve than surface electrodes applied to the skin
(Kimura 1989b). Stimulating electrodes consist of a cathode (negative pole) and an anode
(positive pole). Monopolar or bipolar electrical stimulation of the nerve can be
performed, although the conduction velocities obtained by the two methods differ
considerably. Most clinical studies use bipolar stimulation (Kimura 1989b). As the
current flows between anode and cathode, negative charges that accumulate under the
cathode depolarize the nerve. Conversely, at the same time, positive charges under the
anode hyperpolarize the nerve. In bipolar stimulation, an anodal conduction block is
avoided by placing the cathode closer to the recording site. An electromyographic
stimulator is used to regulate the amount of current that is administered to stimulate a
nerve. Constant-voltage stimulators are used to regulate the output in voltage, so that the
actual current varies inversely with the impedance of electrode, skin, and subcutaneous
tissues. In constant-current units, the voltage changes according to the impedance, so that
a specified amount of current reaches the nerve within certain limits of the skin’s resistance (Kimura 1989b). Electric stimuli can be classified on the basis of the magnitude of the evoked action potential. A threshold stimulus elicits a response in some, but not all axons contained in the nerve. A maximal stimulus activates all groups of axons and a further increase in stimulus intensity causes no additional increase in the amplitude of the evoked potential.

Surface and needle electrodes can be used to record evoked muscle and nerve potentials. Needle electrodes are preferred by some investigators to record sensory and mixed nerve action potentials because the amplitude of the recorded potential increases and the noise from the electrode tissue surface is decreased by a factor of two to three times over surface electrodes (Rosenfalck 1978). Ground electrodes are usually placed subcutaneously between stimulating and recording electrodes.

The potentials assessed during electromyographic procedures range in amplitude from microvolts to millivolts and an amplifying system using a storage oscilloscope or an electromyographic computer is usually required to record them (Kimura 1989b).

The most important parameters obtained by electromyographic diagnostic techniques include latency, amplitude of the evoked compound action potential, and conduction velocity. Latency is referred to as the interval between the onset of a stimulus to a nerve and the onset of the evoked response. Sensory nerve latency is only dependent on nerve conduction properties, whereas motor latency includes neuromuscular transmission (Kimura 1989b). The conduction velocity is the speed of propagation of an action potential along a nerve or muscle fiber (Kimura 1989b). For a nerve trunk, the maximum conduction velocity is calculated from the latency of the evoked potential at
maximal or supramaximal intensity of stimulation at two points. The distance between these two points (between stimulation and recording electrodes or between two different sets of recording electrodes along the nerve trunk) is the conduction distance and is divided by the difference between the corresponding latencies (conduction time) to obtain the conduction velocity (Kimura 1989b). The calculated velocity represents the conduction velocity of the fastest fibers and is expressed in meters per second (m/s). The compound nerve action potential is the summation of nearly synchronous nerve fiber action potentials recorded from a nerve trunk (Kimura 1989b). It is produced by direct or indirect stimulation of the nerve and its amplitude is measured from the most positive to the most negative peak. Under standardized circumstances, shape and amplitude of an evoked compound action potential can be indicative of axonal damage or dysfunction in a specific subset of nerve fibers (Kimura 1989b).

The validity of a calculated nerve conduction velocity depends on a variety of technical factors and influences from the subject. Accuracy in determining the latencies and the correct conduction distance are most imperative to obtain correct measurements. Other sources of error associated with the technique include incorrect triggering of the stimulus, poorly defined takeoff of the evoked response, inappropriate stimulus strength, and inaccurate calibration (Kimura 1989b; Simpson 1964). In people, nerve conduction is faster at warmer body temperatures (De Jesus et al. 1973). Alterations in nerve conduction velocities related to limb temperatures were also identified in horses (Wheeler 1989). In people, both motor and sensory nerve fibers have furthermore been shown to conduct substantially more slowly in legs than in arms, which has been associated with a longer overall length of the nerves (Campbell et al. 1981; Kimura 1989b). One study
comparing horses and ponies demonstrated a consistent inverse relationship between sensory nerve conduction velocities and animal height and nerve segment length (Blythe et al. 1988).

A clear association between age and conduction velocities has been demonstrated in people. Nerve conduction velocities nearly double their values between birth and 3 to 5 years of age, thereby paralleling the process of advancing myelination (Thomas and Lambert 1960). As a function of age and growth in length, both motor and sensory nerve conduction velocities tend to slightly increase in the upper limb and decrease in the lower limb in later childhood and adolescence from age 3 to 19 years of age (Lang et al. 1985). Conduction velocities begin to decline after 30 to 40 years of age, but the values normally change by less than 10 m/s at 60 years of age (Taylor 1984). Studies in horses revealed an increase of sensory nerve conduction velocity over the first year of life followed by a subsequent decrease in older horses (Wheeler 1990).

The type of electromyographic abnormality is usually indicative of a specific kind of lesion in the affected nerve. Substantial slowing in conduction velocity implies demyelination of the sensory nerve fibers, whereas axonal damage with loss of continuity and Wallerian degeneration of the distal nerve segment (axonotmesis) results in reduced amplitude of the CAP with stimulation distal or proximal to the site of the lesion, or even total loss of response (Kimura 1989b).
CHAPTER 3

FUNCTIONAL AND MORPHOLOGICAL CHANGES IN PALMAR DIGITAL NERVES FOLLOWING NON-FOCUSED EXTRACORPOREAL SHOCK WAVE APPLICATION IN HORSES

3.1 Introduction

Extracorporeal shock wave therapy (ESWT) is a newly adapted treatment modality used to treat musculoskeletal disorders in horses (McClure and Merritt 2003). Various case series report successful treatment of soft tissue and bone disorders such as proximal suspensory desmitis (Boening et al. 2000; Crowe et al. 2002), dorsal metacarpal disease (Palmer 2002), navicular disease (Baer et al. 2001; Garcia Lineiro and Echezzareta 2001), and osteoarthritis of the tarsometatarsal and distal intertarsal joints (McCarrol and McClure 2000) with ESWT in horses. Although there is a paucity of controlled studies and the specific mechanism by which shock waves affect tissues still need to be elucidated, ESWT has become increasingly popular in equine practice.

Extracorporeal shock waves (ESW) are pressure gradient waves with a rise time of 5 to 10 ns and a peak pressure of up to 100 megapascals (MPa), followed by a rapid fall to negative pressure before returning to baseline in a total pulse time of approximately 300 ns (Sturtevant 1996). Wave energy is released at interfaces of tissues that have different acoustic impedances, resulting in compression and shear loads on the surface of the material with the greater impedance value. These loads result in a process called cavitation via development and collapse of microscopic gas bubbles. The collapsing gas bubbles invert and a small jet of liquid impinges on the surface of the material with greater impedance, thereby generating extremely high localized stresses (Sturtevant 1996).
Two fundamentally different techniques are used to generate ESW. Focused shock wave generators were originally developed for the non-invasive destruction of urinary calculi in people (Chaussy et al. 1980) and were later adapted to treat a variety of musculoskeletal disorders (Haupt 1997; Rompe et al. 2001). Focused shock wave generators initiate a pressure wave within a fluid medium and the wave is focused by reflection within the generator toward a focal point in the patient (Sturtevant 1996). Non-focused shock waves or radial pressure waves are generated by mechanical concussion. They are characterized by lower energies, a slower rise time than focused waves, and a negative component that is of the same order as the positive component. Their maximum wave energy is observed at the applicator-skin interface and declines rapidly in proportion to the distance from the generator (McClure and Merritt 2003).

Treatment with both focused and non-focused ESW has been reported to cause analgesia. This analgesic effect is most likely independent of any other potential beneficial effects on tissue healing and is observed rapidly after treatment. Clinical case studies in man (Haupt 1997; Rompe et al. 2001) and horses (Boening et al. 2000; McCarrol and McClure 2000) report substantial clinical improvement of various painful orthopedic conditions without concurrent radiographic changes.

Analgesia of an injured limb represents a concern in an equine athlete because it disables protective limiting mechanisms and may place horses with predisposing lesions at an increased risk of sustaining a catastrophic potentially career-ending or life-threatening injury when exercised with altered peripheral pain perception. This has been recognized by the equine industry and has led to the development of safety regulations
concerning the use of ESWT prior to competition or training by racing jurisdictions and the Federation Equine International (McClure and Merritt 2003).

Extracorporeal shock wave therapy does not appear to be used to specifically treat peripheral nerves in man. In horses, nerves of the distal limb or structures in their immediate proximity are often treated directly or indirectly in order to provide relief from painful syndromes in the foot, such as navicular disease (Baer et al. 2001; Garcia Lineiro and Echezzareta 2001). There are no reports regarding the effects of ESWT on function and morphology of peripheral nerves in horses.

The objectives of this study were to demonstrate the post-treatment analgesic effect of ESWT by comparing sensory nerve conduction velocities (SNCV) in treated and untreated peripheral nerves in vivo and to document morphologic changes in ESWT-treated nerve segments by histology and transmission electron microscopy (TEM) after palmar digital neurectomy. It was hypothesized that a single treatment with non-focused ESW applied directly over a peripheral nerve would result in decreased SNCV and noticeable morphologic changes.

3.2 Materials and Methods

3.2.1 Animals

The study was approved by the Institutional Animal Care and Use Committee of the Louisiana State University. A total of six horses were used in. Two Quarterhorses and four Thoroughbred horses (4 geldings, 2 mares) with mean bodyweight of 522 kg (range 464 to 584 kg) and mean age of 14 years (range 5 to 17 years) were evaluated. Prior to inclusion in the study, animals were clinically evaluated and determined to be free of lameness. Horses were selected from a pool of animals used for research; individual
housing was provided at a nearby research facility. All horses were fed a routine complete pelleted diet and had free access to water.

### 3.2.2 Extracorporeal Shock Wave Therapy

All six horses were treated with non-focused ESW on day 0 of the study. Prior to application of the ESW regimen, each horse was sedated with detomidine hydrochloride\(^a\) (0.02 mg/kg of body weight, IV) and butorphanol tartrate\(^b\) (0.02 mg/kg of body weight, IV). The hair on the palmar pastern of both front limbs was clipped and after application of a coupling gel provided by the manufacturer, 2,000 pulses with a non-focused shock wave generator\(^c\) were applied over the medial palmar digital nerve of the left forelimb. The regimen was administered at a frequency of 240 pulses/min., with a machine pressure of 0.25 MPa, using a 15-mm diameter applicator head. The same treatment was then administered to the left lateral palmar digital nerve. No treatment was administered to the right forelimb. The animals were returned to their stalls and daily hand-grazing was provided until subsequent experimental procedures.

### 3.2.3 Sensory Nerve Conduction Velocity Measurements

Three groups of two horses each were evaluated at 3, 7, and 35 days following ESW application, respectively. The animals were sedated with xylazine hydrochloride\(^d\) (0.5 mg/kg of body weight, IV) and butorphanol tartrate\(^b\) (0.02 mg/kg of body weight, IV) and general anesthesia was induced with ketamine hydrochloride\(^e\) (2 mg/kg of body weight, IV) and diazepam\(^f\) (0.15 mg/kg of body weight, IV). Horses were placed in right lateral recumbency and anesthesia was maintained with isoflurane\(^g\) and oxygen in a semi-closed system. The pastern area and fetlock of both front limbs were aseptically prepared and draped, and a lateral abaxial 3-cm incision was made along the deep digital flexor
tendon in the mid-pastern area. The lateral palmar digital nerve was carefully isolated. A second 1-cm incision was made abaxially at the level of the lateral proximal sesamoid bone and the nerve was isolated at this site. A pair of sterile needle electrodes was placed directly into the nerve at the distal incision (stimulating electrodes) and at the proximal incision (recording electrodes). A ground electrode was placed dorsally into the skin between the stimulating and recording electrodes (Figure 3.1). The stimulating electrode was activated by a stimulator from an electromyography system\(^b\) (Figure 3.2). The recording and ground electrodes were connected to the preamplifier of the electromyographic recorder. The distance between stimulation and recording electrodes was measured with a sterile measuring tape and entered into the system computer. Square wave stimuli of 100 µs at 1 Hz were generated by the stimulator at various voltage settings until a visible compound action potential (CAP) was recorded by the recording electrodes and displayed on the system monitor (Figure 3.3). An average of 10 responses per nerve was recorded and measurements in each nerve were conducted in duplicate to demonstrate reproducibility. Sensory nerve conduction velocity (SNCV) in m/s was then calculated by the system computer and the average of both measurements for each nerve was recorded. Subsequently, the left medial and right medial palmar digital nerves were approached similarly through medial abaxial incisions and SNCV was measured as described above.

3.2.4 Palmar Digital Neurectomy

Immediately after the SNCV measurements, a 4-cm long segment of the palmar digital nerve was resected and divided transversely with a sharp scalpel blade. Representative sections were placed in 10% neutral-buffered formalin for histological
evaluation and in primary fixative for transmission electron microscopy (1.25% glutaraldehyde and 2% formaldehyde in 0.1M cacodylate buffer). The incisions were closed with single interrupted skin sutures, using size 2-0 nylon suture and bandaged.

Figure 3.1 - Photograph obtained during sensory nerve conduction velocity measurements in the left lateral palmar digital nerve in a horse under general anesthesia. The foot of the animal is draped off on the left side of the photo. Needle electrodes in contact with the nerve can be observed in the distal (S = stimulating electrodes) and proximal (R = recording electrodes) incisions.

Figure 3.2 - Position of the electrodes placed over the palmar digital nerves of horses for sensory nerve conduction velocity measurements. S = stimulating electrode, R = recording electrode, G = ground electrode, EMG = electromyography system, STIM = electromyographic stimulator.
After recovery from anesthesia, the horses were returned to their stalls. The incision sites were kept bandaged until suture removal at two weeks after surgery. No complications from the surgery were observed.

3.2.5 Histological Examination

Longitudinal and transverse sections of harvested nerve segments were fixed in 10% neutral-buffered formalin, routinely embedded in paraffin, cut at a thickness of 4 µm on a rotary microtome, and stained with hematoxylin and eosin (H&E) and luxol fast blue (LFB). Morphologic changes were described and each nerve was assigned a subjective histological score. Changes were described as mild (grade 1), moderate (grade 2), and severe (grade 3) when the number of affected nerve fibers was less than 10%, between 10% and 40%, and more than 40%, respectively, in the examined sections. Sections with no microscopic findings were assigned grade 0. The pathologist was blind to the origin of the sample (treated vs. control nerves).

3.2.6 Transmission Electron Microscopy

Representative 3-mm² transverse sections of excised nerve segments were washed twice for 15 minutes in 0.1 mol/L sodium cacodylate containing 5% (W/V) sucrose. The buffer wash was followed by post-fixation in 1% OsO₄ in distilled water for 1 hour. After several washes with distilled water, the nerve sections were stained overnight in 0.5% (W/V) uranyl acetate in distilled water. The samples were then dehydrated in graded ethanol solutions of increasing concentrations of 30%, 50%, 70%, 95% and 100%, infiltrated with Poly/Bed 812, and polymerized. Polymerized blocks were sectioned into 0.1-µm sections on an ultramicrotome. The sections were stained with uranyl acetate and
lead citrate, and examined using a transmission electron microscope. The investigator was blind to the origin of the sample (treated vs. control nerves).

3.2.7 Data Analysis

SNCV was considered continuous and the proportional difference in SNCV for treated lateral and medial nerves, compared with control nerves within each horse, was analyzed using a mixed effect linear model that accounted for random variance of horse and limb, nested within time points. Ad-hoc comparisons were made within each nerve and across treated nerves at each time point, maintaining type I error at 0.05. Thus, where significance was determined, the p-value was set at ≤ 0.05, unless specified. A software program was used for analysis.

3.3 Results

3.3.1 Sensory Nerve Conduction Velocities

Velocities for treated and control nerves for each horse were summarized (Table 3.1). Careful surgical exploration and identification of the palmar digital nerves prior to needle placement for stimulating and recording electrodes resulted in reliable and reproducible SNCV measurements (Figure 3.3). Non-focused ESW caused a significant decrease in SNCV in all treated nerves, compared with control nerves, at days 3 and 7. A significant decrease in SNCV was also observed in treated medial but not lateral nerves at day 35. There was no difference in the magnitude of decrease between treated medial and lateral nerves on days 3 and 7.

3.3.2 Histological Examination

Varying degrees of segmental axonal swelling were the only histological change observed. Swollen axonal segments had a pale, fine granular texture and were often
Figure 3.3 - Compound action potential (CAP) recorded from a palmar digital nerve of a horse after electrical stimulation. O = onset, P = peak, T = through. Arrow = stimulus artifact.

Table 3.1 - Mean (individual values) of sensory nerve conduction velocities (m/s) and individual subjective histological severity scores in palmar digital nerves of horses treated with non-focused extracorporeal shock waves at 3, 7, and 35 days after treatment. SNCV = sensory nerve conduction velocity, HSS = histological severity score, LL = left lateral palmar digital nerve, LM = left medial palmar digital nerve, RM = right medial palmar digital nerve (untreated control). There was no difference in magnitude of the decrease of SNCV for LL and LM nerves at days 3 and 7. Within each time point, “a” denotes a significant decrease in SNCV compared to controls (RM).

<table>
<thead>
<tr>
<th>Day post treatment</th>
<th>Nerve</th>
<th>SNCV</th>
<th>HSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>LL</td>
<td>32.45a</td>
<td>0, 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(32.7, 32.2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LM</td>
<td>32.15a</td>
<td>2, 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(34.9, 30.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RM</td>
<td>48.2</td>
<td>2, 0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(49.9, 46.5)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>LL</td>
<td>37.0a</td>
<td>2, 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(39.1, 34.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LM</td>
<td>32.26a</td>
<td>3, 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(34.9, 30.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RM</td>
<td>60.25</td>
<td>2, 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(61.1, 59.4)</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>LL</td>
<td>51.95</td>
<td>0, 0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(61.7, 42.2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LM</td>
<td>37.9a</td>
<td>2, 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(37.4, 38.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RM</td>
<td>43.35</td>
<td>2, 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(44.7, 42.0)</td>
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</tr>
</tbody>
</table>
Figure 3.4 - Photomicrograph of a longitudinal section of the left medial palmar digital nerve of a horse at 7 days after treatment with non-focused ESW (grade 3 histological severity score). Note the fine granular texture in swollen axonal segments (arrow) and the decreased cellularity of Schwann cells. Similar changes were observed in treated nerves and untreated control nerves. Bar = 50 µm, luxol fast blue stain.

Figure 3.5 - Photomicrograph of a longitudinal section of the untreated right medial palmar digital nerve (control nerve) of a horse at 3 days after treatment of the nerves on the opposite limb with non-focused extracorporeal shock wave therapy (grade 0 histological severity score). No changes can be observed in myelinated (arrows) and unmyelinated nerve fibers and in surrounding Schwann cells. Bar = 50 µm, hematoxylin and eosin stain.
localized at only one end of longitudinal sections. No cellular infiltrates or hemorrhage was noted. Changes were observed in most treated and untreated nerve segments at 3, 7, and 35 days after ESW application (Table 3.1). Severely affected nerve segments had a decreased cellularity. The most severe changes were observed in left medial nerves at 7 days after ESW application (Figure 3.4). Some of the control nerve samples and some of the treated nerve samples revealed no detectable changes (Figure 3.5).

### 3.3.3 Transmission Electron Microscopy

Transverse sections of treated and untreated nerve segments were examined. Large- to medium-sized and small myelinated axons were observed. Non-myelinated axons were surrounded by neurolemmacytes and embedded in a loose collagenous endoneurium. In untreated nerves, concentric uniform myelin sheaths were evident in all myelinated axons and no traumatic or inflammatory changes were observed (Figure 3.6). Treated nerves at 3 (Figure 3.7), 7 (Figure 3.8) and 35 (Figure 3.9) days after ESWT revealed extensive separation and disruption between the different layers of the myelin sheath in large- to medium-sized myelinated axons, but no changes were observed in small myelinated and non-myelinated axons. As with untreated nerves, no traumatic or inflammatory changes were observed.

### 3.4 Discussion

This study revealed that a single application non-focused ESW over a peripheral nerve results in a considerable decrease in conduction velocity and in separation and disruption of the layers of the myelin sheath in large- to medium-sized myelinated axons. A significant proportional difference in SNCV of ESW-treated versus untreated control
Figure 3.6 - Photomicrograph obtained by transmission electron microscopy of a transverse section through an untreated control nerve. Large- to medium-sized (dark arrowheads) and small (light arrowheads) myelinated axons and numerous unmyelinated axons (dark arrows) can be observed. Bar = 10 µm.

nerves was observed at 3 and 7 days following ESW treatment. Treated medial, but not lateral, nerves also had a significantly decreased SNCV at 35 days following ESW application.

It is believed that the absence of electrophysiologic changes in treated lateral nerves at 35 days after treatment is most likely attributable to a lack of precision during application of the ESW regimen and that the effects of ESW application on conduction in peripheral nerves could most likely last beyond the follow-up period of the present
Figure 3.7 - Photomicrograph obtained by transmission electron microscopy of a transverse section through the left medial palmar digital nerve of a horse at 3 days after treatment with non-focused extracorporeal shock wave therapy. There is separation between the myelin layers in large-to medium-sized myelinated axons (dark arrowheads). No changes can be observed in small myelinated axons (light arrowheads) and unmyelinated axons (dark arrows). Bar = 10 µm.

Study. Transmission electron microscopy consistently revealed separation and disruption of the layers of the myelin sheath of large- to medium-sized myelinated axons in treated nerves.

Histology revealed varying degrees of axonal swelling and cytoplasmic dissolution with a fine granular texture in both treated and control nerve samples at all time points. The histological changes have therefore to be considered artifacts that occurred most likely during the sensory SNCV measurements or during the subsequent palmar digital neurectomy.
Figure 3.8 - Photomicrograph obtained by transmission electron microscopy of a transverse section through the left medial palmar digital nerve at 7 days after treatment with non-focused extracorporeal shock wave therapy. Severe disruption is evident in the myelin sheath of large- to medium-sized myelinated axons (dark arrowheads). There are no changes in small myelinated (light arrowheads) and unmyelinated axons (dark arrows). Bar = 10 µm.

Results of nerve conduction studies closely parallel the structural abnormalities of the nerve and depend on the type and degree of nerve damage. Partial demyelination and traumatic damage to the myelin sheath in myelinated axons has been shown to result in an impaired saltatory conduction due to an increase in internodal capacitance and conductance (Kimura 1989b). More local current is lost to charge the membrane capacitance and by leakage through the internodal membrane before reaching the next
Figure 3.9 - Photomicrograph obtained by transmission electron microscopy of a transverse section through the left medial palmar digital nerve at 35 days after treatment with non-focused extracorporeal shock wave therapy. Large- to medium-sized myelinated axons (dark arrowheads) reveal similar changes as in Figure 3.8. There are no changes in small myelinated fibers (light arrowheads) and unmyelinated fibers (dark arrows). Bar = 10 µm.

node of Ranvier. Thus, axons with a damaged myelin sheath characteristically exhibit decreased conduction velocity and temporal dispersion (Kimura 1989b). Impaired nerve conduction without actual structural damage to the axonal cytoplasm is referred to as neuropraxia and represents the mildest form of peripheral nerve injury (Seddon 1943).

The electron microscopic findings could explain the decrease in SNCV values obtained in shock wave-treated nerves in the present study.
The lack of electron microscopic changes in unmyelinated and small-sized myelinated axons in treated nerves was consistent and it may be speculated that these structures are either too small or provide an insufficient local change in impedance for ESW to exert their effects.

Lameness disorders represent a therapeutic challenge to the equine practitioner who wants to provide pain relief, reinstitute athletic use of the horse, and minimize economic loss while operating within ethical and regulatory constraints of modern competition. This has led to a growing interest in new, alternative treatment modalities that appear to result in an improved and accelerated healing process and a shorter convalescence period. Extracorporeal shock wave therapy represents such a modality and appears to be gaining interest and acceptance in the equine industry among veterinarians, trainers and owners for therapy of selected orthopedic injuries in horses.

Shock wave-induced stimulation of bone and soft tissue healing has been observed in man (Haupt 1997; Wild et al. 2000) and various animal species (Johannes et al. 1994; McClure and Merritt 2003; Wang et al. 2002), however, the exact effects of high pressure waves on tissues are not yet fully understood. Treatments with focused and non-focused ESW also seem to cause a transient analgesic effect within treated areas, apparently independent of any other beneficial effect on tissues (McClure and Merritt 2003). The exact mechanism of this analgesic effect is unknown. Haist and von Keitz-Steeger (1995) postulated three hypotheses for how shock waves induce analgesia in humans: 1) shock waves induce cell damage and the peripheral nociceptors can therefore not build up a membrane potential sufficient to transmit pain signals; 2) the nociceptors are overstimulated by shock waves and emit high frequency impulses to peripheral nerve
fibers, which are suppressed by a gate control mechanism; and 3) shock wave-induced pericellular free radicals induce the local release of unknown pain-suppressing substances (Haist and von Keitz-Steeger 1995).

In man, the analgesic response following ESWT appears to be bimodal. An immediate initial decrease in pain for 3 to 4 days is followed by a return of the pain and then a second gradual reduction in pain that occurs over the ensuing 3 to 4 weeks (Ogden and Ogden 2002). The initial response has been attributed to the direct effects of ESW on nociceptors and impaired substance P synthesis; whereas the second phase of pain relief was believed to result from a healing response associated with angiogenesis and tissue matrix remodeling (Ogden and Ogden 2002). A similar bimodal analgesic response can be observed clinically in horses undergoing ESWT to treat musculoskeletal disorders (McClure and Merritt 2003).

Musculoskeletal activity without full perception of peripheral pain could potentially place equine athletes with predisposing lesions at an increased risk of sustaining career-ending or life-threatening injuries such as condylar fracture of the third metacarpus or metatarsus, or breakdown injuries of the suspensory apparatus. This risk has been recognized by the equine industry and strict regulations concerning the use of ESWT before competitions have been issued by multiple racing jurisdictions and the Federation Equine International (McClure and Merritt 2003).

It has been recommended to avoid large nerves, blood vessels, and active growth plates when applying ESWT to horses (McClure and Merritt 2003). Because of the anatomical proximity of the target tissues to these structures, nerve trauma, however, cannot always be avoided, or it is occasionally even desired (Garcia Lineiro and
Echezzareta 2001). In horses, the palmar digital nerves may be treated directly or indirectly when ESWT is used to treat disorders in the foot, such as navicular disease (Baer et al. 2001; Garcia Lineiro and Echezzareta 2001). Extracorporeal shock wave therapy does not appear to be used to specifically treat peripheral nerves in humans.

This study does not propose a conclusive explanation for the analgesic effect observed subsequent to ESWT in horses; however, it supports the premise that ESW cause damage to peripheral nerves resulting in slower conduction velocities and potentially impaired perception of peripheral pain.

Morphological and functional changes associated with neuropraxia are reversible over time (Kimura 1989b). Repeated application of ESWT, however, may cause more extensive damage to exposed peripheral nerves and may result in prolonged or permanent alterations in nerve conduction. Renal damage following extracorporeal shock wave lithotripsy (ESWL) of nephroliths in dogs has been shown to be cumulative depending on dose (voltage and number of shock waves) and frequency (number of shock waves per second) of the administered regimen (Lingeman et al. 1989; Newman et al. 1987). An experimental study also revealed dose-dependent morphological damage in the Achilles tendon of rabbits after application of ESW (Rompe et al. 1998). It may be speculated that administration of a greater total dose regimen of ESW may have resulted in prolonged duration of impaired nerve conduction and more extensive morphological damage in treated peripheral nerves. The duration of morphological nerve damage and impaired nerve conduction was not assessed beyond 35 days in the present study. The results of this study indicate that further research would be necessary to assess the effects of non-
focused extracorporeal shock waves on peripheral nerves after a longer time period and after multiple treatments.

In conclusion, direct treatment of palmar digital nerves in horses with a single application of non-focused ESW resulted in a decrease of nerve conduction velocity and distinct alterations in the myelin sheath of large- and medium-sized myelinated axons. Although the small sample size of the present study limits the ability to draw a strong conclusion and despite the fact that the results do not provide a conclusive explanation for the analgesic effect observed in a clinical situation, cautious use of this treatment regimen is recommended in equine athletes before training or competition.

3.5 Product Information

aDormosedan, Pfizer, Inc., New York, NY.
bTorbugesic, Fort Dodge Animal Health, Fort Dodge, IA.
cSwiss ColorClast® Vet, EMS Electro Medical Systems, Dallas, TX.
dXylaxine 100 Injection, The Butler Company, Columbus, OH.
eKetaset, Fort Dodge Animal Health, Fort Dodge, IA.
fDiazepam Injection, Abbott Laboratories, North Chicago, IL.
gIsoFlo, Abbott Laboratories, North Chicago, IL.
hCadwell Sierra EMG/EP, Cadwell Laboratories, Kennewick, WA.
iEthicon, Johnson & Johnson, Somerville, NJ.
jShandon AS325, Shandon Products, Pittsburgh, PA.
kPoly/Bed 812, Polyscience, Inc., Warrington, PA.
lMT-XL, RMC Products by Boeckeler Instruments, Inc., Tucson, AZ.
mZeiss (LEO) EM-10C, Oberkochen, Germany.
nPROC MIXED, SAS V8.0, SAS Institute, Inc., Cary, NC.
CHAPTER 4
EVALUATION OF THE LOCAL ANALGESIC EFFECT AFTER NON-FOCUSED EXTRACORPOREAL SHOCK WAVE APPLICATION TO THE THIRD METACARPAL BONE IN HORSES

4.1 Introduction

Periostitis and stress fractures of the dorsal cortex of the third metacarpal bone represent conditions that are often observed in young fast-gaited performance horses at two to three years of age (Bertone 2002). This disease complex is referred to as dorsal metacarpal disease (DMD) and is believed to occur because of a failure of the adaptive response to altered biomechanical stresses that arise from high-speed work (Bertone 2002). Racing Thoroughbreds have the greatest prevalence of DMD, but young Quarter Horses and occasionally racing Standardbreds can also be affected (Bertone 2002).

Conventional treatment of DMD includes combinations of stall rest, altered training regimens, and systemically administered non-steroidal anti-inflammatory drugs (NSAIDs), percutaneous periosteal scraping, and osteostixis. Alternative treatment modalities, such as cryotherapy (Montgomery et al. 1981), and more recently, extracorporeal shock wave therapy (Palmer 2002; Scheuch et al. 2000), have also been reported for the treatment of DMD. In a clinical report on 50 Thoroughbred racehorses with DMD that were unresponsive to conventional treatment and were treated with pneumatically generated non-focused extracorporeal shock wave therapy (ESWT), 40 horses (80%) resumed speed work without recurrence of lameness or other clinical signs associated with DMD after three treatments at three-week intervals (Palmer 2002). In another case series, 10 horses with dorsal or palmar metacarpal cortical stress fractures were treated with one to three applications of focused ESWT administered under
extracorporeal shock waves are acoustic pressure gradient waves that are used to treat a variety of musculoskeletal disorders in people and animals. Although first introduced for non-invasive treatment of urolithiasis in man (Chaussy et al. 1980), ESW were subsequently investigated for their effect on wound healing (Haupt and Chvapil 1990) and their osteogenic potential (Ikeda et al. 1999; Johannes et al. 1994). Humans are currently treated with ESWT for a variety of conditions including fracture non-unions (Haupt 1997; Valchanou and Michailov 1991) and painful soft tissue syndromes such as plantar calcaneal spurs (Rompe et al. 2003) and tennis elbow (Rompe et al. 2001). Several clinical investigations in horses reported promising results in the treatment of a variety of disorders such as bone spavin (McCarrol and McClure 2000), stress fractures (Scheuch et al. 2000), high suspensory desmitis (Boening et al. 2000; Crowe et al. 2002) and navicular disease (Baer et al. 2001; Garcia Lineiro and Echezzareta 2001). Horses are often treated using non-focused ESW or radial pressure waves that are characterized by their distinct physical characteristics including lower wave energies, a slower rise time, and their inferior penetration into the patient’s body compared to focused ESW (McClure and Merritt 2003). However, beneficial effects in treatment of musculoskeletal disorders with non-focused ESW have been reported in people (Gremion et al. 2001; Lohrer et al. 2001a; Lohrer et al. 2001b) and horses (Boening et al. 2000; Crowe et al. 2002; Palmer 2002).
Although the precise mechanism by which ESW exert their effect on tissues are currently not known, transient local analgesia of the treated areas has been observed in people (Ogden and Ogden 2002) and horses (McClure and Merritt 2003). This analgesic effect is likely to be independent of any other beneficial effect on healing of injured structures and could place performance horses with predisposing bone and soft tissue lesions at risk of sustaining a life- or career-threatening injury when exercised too strenuously after application of shock wave therapy. Recognition of these safety concerns has resulted in the development of regulations concerning the use of ESWT prior to competition and training by racing jurisdictions and the Federation Equine International (McClure and Merritt 2003).

The purpose of this study was to evaluate the local cutaneous analgesic effect of ESW in the distal limb of horses. It was hypothesized that a single treatment with non-focused ESW applied dorsally to the third metacarpal bone would induce a transient local analgesic effect and that altered peripheral pain perception could be assessed by evaluating the response to a standardized thermal cutaneous pain stimulus. The specific objective was to compare the latency of onset, magnitude and duration of analgesia in metacarpal areas treated with non-focused ESW to non-treated areas.

4.2 Materials and Methods

4.2.1 Animals

The study was approved by the Institutional Animal Care and Use Committee of the Louisiana State University. Twelve adult horses from the institutional research herd were used in the study: Five Quarter Horses (three geldings, two mares), six Thoroughbreds (four geldings, two mares) and one Arabian with a mean age of 14 years.
(range five to 19 years). Prior to inclusion in the study, all animals were clinically evaluated and determined to be free of lameness and apparent abnormalities in their metacarpal bones. Individual housing was provided at a nearby research facility and all horses were fed a routine complete pelleted diet and had free access to water.

4.2.2 Extracorporeal Shock Wave Therapy

Prior to ESW application, each horse was sedated with detomidine hydrochloride (0.02 mg/kg of body weight, IV) and butorphanol tartrate (0.02 mg/kg of body weight, IV) and the hair on the medial, lateral and dorsal aspect of both metacarpi was clipped. One forelimb of each animal was randomly assigned to be treated. The skin of five circular areas (1.5 cm diameter) on the treated metacarpus (lateral-mid-diaphysis = area 1; dorsal-mid-diaphysis = area 2; medial-mid-diaphysis = area 3; dorsal-proximal diaphysis = area 4; dorsal-distal diaphysis = area 5) and on one area on the control limb (dorsal metacarpal mid-diaphysis = area 6) of each animal were darkened with a black ink felt pen to improve light absorption for assessment of the limb withdrawal reflex latency (LWRL). After application of a coupling gel provided by the manufacturer, 2,000 pulses with a non-focused ESW generator were applied over each of the three mid-diaphyseal circular areas of the treatment limb (areas 1, 2, 3). The regimen was administered at a frequency of 240 pulses/min., with a machine pressure of 0.25 MPa, using a 15-mm diameter applicator head. The proximal and distal diaphyseal areas on the treated limb (areas 4 and 5) and the mid-diaphyseal area on the opposite limb (area 6) served as controls (Figure 4.1).
Figure 4.1 - Areas for evaluation of the limb withdrawal reflex latency (LWRL) on the third metacarpal bones of horses treated with non-focused extracorporeal shock waves. A = shock wave-treated limb. B = untreated control limb. 1, 2, 3 = treated areas. 4, 5, 6 = untreated control areas.

4.2.3 Assessment of the Limb Withdrawal Reflex Latency

The degree of cutaneous analgesia caused by non-focused ESW was quantified by assessment of alterations in a nociceptive threshold, using a focused light beam as a stimulus. Briefly, horses were led into the testing area and a focused beam of light of a standardized intensity was emitted from a distance of 20 cm onto the target areas on the third metacarpal bones. The time from stimulus when the light beam was emitted until withdrawal of the limb was observed, and this response was designated as the limb withdrawal reflex latency (LWRL). Baseline measurements were obtained in both treated
and control areas prior to application of the ESW regimen. Following treatment of the designated areas, LWRL was assessed in random order in all areas of both limbs every two hours for 12 hours, followed by measurements every eight hours for 48 hours after treatment. Each LWRL measurement was repeated three times in each area.

4.2.4 Data Analysis

Time to withdrawal from the light source was considered continuous, and determined to follow a normal distribution, using the Shapiro-Wilk test with rejection of the null hypothesis of normality at \( p \leq 0.05 \). Results were summarized as mean ± SEM. Using each horse as its own control (blocking by horse), the effects of treatment and time were evaluated, using a mixed effect linear model accounting for the random variance of horses and repeated measurements on each horse. Where there were significant main and interaction effects, selected multiple comparisons were made to determine the between treatment effects at various time points and the effect of time within treatments. A software program was used for analysis.

4.3 Results

Application of non-focused ESW was well tolerated by all horses. Eight of the 12 horses developed mild to moderate swelling and skin abrasions over the treated areas. These changes were observed immediately after the end of ESW application.

The LWRL responses were similar in all treated and control areas over time with a significant decrease noted at most areas and time points compared with baseline measurements.
Table 4.1 - Mean ± (SEM) limb withdrawal reflex latency values of horses after application of non-focused shock waves to the metacarpus. Areas 1, 2, 3 = treated areas on mid-diaphysis of the treated limb; areas 4, 5 = untreated control areas on the treated limb; area 6 = mid-diaphyseal area on the opposite limb (untreated control). Within each treatment area (row), “a” denotes means that are significantly (p ≤ 0.05) different from the baseline measurements. Within each time point (column), “b” indicates means that are significantly (p ≤ 0.05) different from area 6 (untreated control).

<table>
<thead>
<tr>
<th>Area</th>
<th>Baseline</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>20</th>
<th>28</th>
<th>48</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13.41 (1.71)</td>
<td>10.76 (0.89)</td>
<td>a</td>
<td>8.96 (0.54)</td>
<td>a</td>
<td>7.76 (0.55)</td>
<td>a</td>
<td>6.93 (0.35)</td>
<td>a-b</td>
<td>6.32 (0.45)</td>
</tr>
<tr>
<td>2</td>
<td>15.18 (1.65)</td>
<td>11.73 (1.36)</td>
<td>10.46 (0.82)</td>
<td>a</td>
<td>8.27 (0.84)</td>
<td>a</td>
<td>7.64 (0.59)</td>
<td>a-b</td>
<td>7.36 (0.62)</td>
<td>a</td>
</tr>
<tr>
<td>3</td>
<td>17.66 (2.54)</td>
<td>12.11 (0.10)</td>
<td>a</td>
<td>9.66 (0.92)</td>
<td>a</td>
<td>8.52 (0.74)</td>
<td>a</td>
<td>8.05 (0.44)</td>
<td>a-b</td>
<td>7.32 (0.46)</td>
</tr>
<tr>
<td>4</td>
<td>13.47 (1.28)</td>
<td>10.89 (0.87)</td>
<td>a</td>
<td>9.17 (0.75)</td>
<td>a</td>
<td>8.30 (0.88)</td>
<td>a</td>
<td>7.28 (0.59)</td>
<td>a-b</td>
<td>7.18 (0.55)</td>
</tr>
<tr>
<td>5</td>
<td>14.79 (1.59)</td>
<td>11.70 (0.42)</td>
<td>a</td>
<td>10.04 (0.80)</td>
<td>a</td>
<td>8.26 (1.13)</td>
<td>a</td>
<td>8.92 (0.80)</td>
<td>a</td>
<td>8.05 (0.67)</td>
</tr>
<tr>
<td>6</td>
<td>17.26 (2.45)</td>
<td>12.43 (0.72)</td>
<td>a</td>
<td>10.84 (1.20)</td>
<td>a</td>
<td>9.36 (0.75)</td>
<td>a</td>
<td>11.06 (1.38)</td>
<td>a</td>
<td>9.53 (0.83)</td>
</tr>
</tbody>
</table>
Between point comparisons at different times after treatment revealed no significant difference between areas during baseline LWRL measurements, and at 2, 4, 6 and 28 hours after ESW application (Table 4.1). There was a significant decrease in LWRL at most areas on the treated limb, compared with the control area on the opposite limb (area 6), at 8, 10, 12, 20 and 48 hours after treatment.

4.4 Discussion

Non-focused ESW applied to the third metacarpal bone of horses had no noticeable effect on LWRL in the present study. A significant decrease in LWRL was observed over time, but there was no difference between treated and control areas on ESW-treated metacarpi and control areas on the opposite limbs. Because swelling and skin abrasions were observed in several horses, a local inflammatory response secondary to treatment-induced trauma could potentially have overwhelmed any local cutaneous analgesic effect. However, considering the similarity of the findings in treated and untreated areas over time, it appears more likely that no local analgesia resulted from ESW application. Tissue trauma and inflammation after ESW application may have induced hypersensitivity in all testing sites of the treated limbs. This could be somewhat corroborated by the significant decrease of LWRL in some areas of the treated limb, compared with the control area on the opposite limb (area 6), at 8, 10, 12, 20 and 48 hours after treatment. Another explanation for the progressive decrease of LWRL in all tested areas over time could be that the horses became conditioned to testing during the study and that a painful thermal stimulus was anticipated. Other studies using the same type of thermal stimulus to assess the analgesic effects of intravenously (Kamerling et al. 1985) and locally (Harkins et al. 1996; Lopez-Sanroman et al. 2003) administered drugs,
and to demonstrate local analgesia after acupuncture and electroacupuncture treatment (Skarda et al. 2002) did not report conditioning of animals to the testing procedure. Preventative measures, such as blindfolding the horses prior to testing (Lopez-Sanroman et al. 2003) or intermittent use of an unfocused (sham) light source (Harkins et al. 1996) have been used in some protocols, however, they were not implemented in the present study.

The effect of ESW application to the third metacarpal bone on LWRL was not assessed beyond 48 hours. This follow-up period was chosen based on clinical observations in humans (Ogden and Ogden 2002) where the analgesic response appears to be biphasic. An immediate initial improvement in clinical signs is normally followed by a return of the pain at three to four days after treatment and then a second gradual improvement over the ensuing 3 to 4 weeks. The initial response is believed to result from the direct effects of shock waves on nociceptors and impaired substance P synthesis, whereas the second phase of pain relief is believed to result from a healing response associated with angiogenesis and tissue matrix remodeling (Ogden and Ogden 2002). A study in sheep, however, failed to identify depletion of the neuropeptides substance P and calcitonin gene-related peptide in the soft tissues overlying metacarpi after treatment with focused or non-focused ESW (McClure et al. 2003).

The results of the present study are contradictory to observations made in another investigation where cutaneous analgesia of the overlying skin during the first 72 hours after application of focused or non-focused ESW to the third metacarpal bone in horses was observed (McClure et al. 2003). In that study, however, cutaneous sensation was
assessed only once every 24 hours and an electric stimulus was used to test for local cutaneous analgesia (McClure et al. 2003).

In people, shock wave therapy is often used to provide relief in painful conditions at the soft tissue – bone interface such as tennis elbow, calcific shoulder tendonitis, or plantar calcaneal spurs (Haupt 1997; Rompe et al. 1996; Rompe et al. 2001). The rapid improvement of clinical signs after ESWT is usually not accompanied by concurrent radiographic changes and has been attributed to an analgesic effect that appears to be independent of the healing response in tissues (Haupt 1997). Similar observations were made in horses with osteoarthritis of the distal intertarsal and tarsometatarsal joints. Eighty percent of the horses improved at least one lameness grade and 18% were completely sound at 90 days after a single treatment with focused ESWT. No concurrent radiographic changes were observed in any of the horses (McCarrol and McClure 2000).

Haist and von Keitz-Steiger proposed three hypotheses for shock wave-induced analgesia in people: 1) shock waves induce cell damage and the peripheral nociceptors can therefore not build up a membrane potential to transmit pain signals; 2) the nociceptors are over-stimulated by shock waves and emit high frequency impulses to peripheral nerve fibers, which are suppressed by a gate control mechanism; and 3) shock wave-induced pericellular free radicals induce the local release of unknown pain-suppressing substances (Haist and von Keitz-Steeger 1995).

Direct effects of ESW on peripheral nerves could contribute substantially to regional analgesia after ESWT. A study in isolated frog sciatic nerves revealed repetitive generation of action potentials during application of ESW (Schelling et al. 1994). Although this in vitro mechanism may not be applicable to the distal equine limb in vivo
(McClure et al. 2003), it is possible that ESW could over-stimulate peripheral sensory nerve fibers and that signal transmission could be impaired by modulation via a gate control-like mechanism (Haist and von Keitz-Steeger 1995). A study in horses revealed a significant decrease in sensory nerve conduction velocity in palmar digital nerves after direct treatment with non-focused ESW compared with untreated control nerves (Bolt et al. 2003). Transmission electron microscopy of treated nerves showed disruption and separation within the myelin sheaths of large- to medium-sized myelinated axons (Bolt et al. 2003). The lack of a cutaneous analgesic effect in the present study could therefore possibly be explained by the absence of peripheral nerves with large myelinated axons in the treated areas.

It has been recommended to avoid peripheral nerves, large blood vessels and growth plates when applying ESWT to horses (McClure and Merritt 2003). However, peripheral nerves are often directly targeted when ESW are used to treat conditions in the distal limb such as navicular disease (Garcia Lineiro and Echezzareta 2001). Although clinical improvement of navicular disease after ESWT has been observed (Baer et al. 2001; Garcia Lineiro and Echezzareta 2001), an experimental study in horses failed to identify local cutaneous analgesia in the heel area following ESW application to the palmar digital nerves in the pastern (McClure et al. 2003).

In conclusion, the findings of the present study show that a single application of non-focused ESW had no noticeable cutaneous analgesic effect in the equine metacarpus. These findings, in light of clinical observations of post-treatment analgesia in people and horses, results of other experimental studies (McClure et al. 2003), and distinct changes in directly treated peripheral nerves after ESW application (Bolt et al. 2003), indicate that
this treatment modality should be used cautiously when treating equine athletes in training or before competition.

4.5 Product Information

\( ^a \)Dormosedan, Pfizer, Inc., New York, NY.
\( ^b \)Torbugesic, Fort Dodge Animal Health, Fort Dodge, IA.
\( ^c \)Swiss DolorClast® Vet, EMS Electro Medical Systems, Dallas, TX.
\( ^e \)PROC UNIVARIATE, SAS V8.0, SAS Institute, Inc., Cary, NC.
\( ^f \)PROC MIXED, SAS V8.0, SAS Institute, Inc., Cary, NC.
CHAPTER 5
SUMMARY

In the first study, application of 2,000 pulses of non-focused extracorporeal shock waves (ESW) to the palmar digital nerves of horses in the pastern area resulted in decreased sensory nerve conduction velocities (SNCV) and caused distinct morphologic changes at 3, 7, and 35 days after treatment. Morphologic changes detected by transmission electron microscopy consisted of an extensive separation and disruption between the different layers of the myelin sheath in large- to medium-sized myelinated axons. These alterations are compatible with decreased SNCV assessed prior to surgical removal of the nerves for morphologic evaluation. No changes were detected in small myelinated and non-myelinated axons. Microscopic evaluation revealed varying degrees of axonal swelling and cytoplasmic dissolution with a fine granular texture in both treated and untreated nerves at all time points. The microscopic changes have therefore to be considered artifacts that occurred most likely during the sensory SNCV measurements or during the subsequent palmar digital neurectomy.

In the second study, no significant difference in nociceptive threshold was found between areas of the metacarpus treated with 2,000 pulses of non-focused ESW and untreated control areas on the same and on the contralateral metacarpus when cutaneous sensation was assessed with a focused light source. Limb withdrawal reflex latency (LWRL) responses in all horses were comparable in treated and control areas over time with a significant decrease noted at most sites and times compared with baseline measurements. Between area comparisons at different times after treatment revealed a decrease in LWRL between most sites of the treated limb and the untreated control area.
of the contralateral metacarpus. These findings may indicate conditioning of the animals to the LWRL testing method, although the decrease in LWRL between most areas of the treated metacarpus and the non-treated area of the opposite metacarpus may be due to a treatment-induced hypersensitivity of the entire dorsal metacarpal surface following application of non-focused ESW.

Although the findings of these studies do not provide a conclusive explanation for the analgesic effect observed after clinical application of ESW, they provide some evidence that ESW can induce distinct electrophysiologic and morphologic changes when they are administered directly to peripheral nerves. Further investigations are required to better understand the analgesic affect after application of ESW in horses and to evaluate the effects of repeated ESW applications on function and morphology of peripheral nerves.
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VITA

David Manuel Bolt was born on May 12, 1968, in Bern, Switzerland. He grew up in Ittigen, Switzerland, and completed his secondary education with a Matura Typus B at the Literargymnasium Kirchenfeld, Bern, Switzerland, in 1987. David attended the University of Bern, Switzerland, earning a med. vet. degree in 1994. He successfully completed his doctoral thesis and earned a Dr. med. vet. degree from the University of Bern, Switzerland, in 1996.

From 1995 to 1996, Dr. Bolt was employed in the Institute of Animal Pathology, University of Bern, Switzerland. From 1997 to 1999, he completed a three-year rotating program in equine medicine, surgery and radiology at the University of Bern, Switzerland. From December 1999 to June 2000, Dr. Bolt was employed as an equine practitioner in Niederlenz, Switzerland. In July 2000, he entered a one-year rotating internship in equine medicine and surgery at the Louisiana State University School of Veterinary Medicine in Baton Rouge, Louisiana. Dr. Bolt is currently completing his final year of a three-year equine surgery residency at Louisiana State University School of Veterinary Medicine in Baton Rouge, Louisiana, and will be awarded the degree of Master of Science in Veterinary Medical Sciences in May 2004.