Effects of pico-tesla electromagnetic field treatment on wound healing in rats

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Objective—To evaluate the effects of a pico-tesla electromagnetic field (PTEF) on healing of sutured and open skin wounds and clinicopathologic variables in rats.

Animals—64 male Fischer-344 rats.

Procedure—An incision made in the dorsal aspect of the neck was sutured (n = 32) or left open to heal (32). In each group, 16 rats were not PTEF-treated (controls). Wound treatment consisted of exposure to a PTEF once daily. Rats in each group were euthanatized at days 2, 4, 7, and 14. Wounds were evaluated via tensiometry (sutured wounds), digital planimetry (open wounds), laser Doppler perfusion imaging, bacteriologic culture, and histologic examination. Blood samples were collected from all rats for analysis.

Results—At day 14, sutured wounds in PTEF-treated rats were stronger (ultimate stress) and tougher (strain energy) than were sutured wounds in control rats. Open wounds in PTEF-treated rats contracted more quickly at days 2 and 4 than did those in control rats. Compared with control wounds, histologic changes (indicative of improved healing) in sutured and open wounds in PTEF-treated rats were detected as early as day 4. Laser Doppler perfusion measurements, results of CBCs, serum biochemical analyses, and bacteriologic cultures were not different between groups.

Conclusions and Clinical Relevance—Exposure to the PTEF caused no adverse effects on clinicopathologic, histologic, or bacteriologic variables tested in this study. It appears that PTEF is a safe form of adjuvant treatment for wounds and improves strength of sutured wounds and speeds contraction of open wounds. (*Am J Vet Res* 2003;64:845–854)

Pico-tesla electromagnetic field (**PTEF**) treatment is based on the premise that weak electromagnetic fields influence biological systems in many ways. Very-low-intensity and extremely-low-frequency electromagnetic fields (in the **pico-tesla [pT]** range) may affect cells by producing a chemical electromagnetic photon-photon transduction or conversion.[1] The phenomenon has been described as a virtual photon flux between the quantum vacuum and ponderable matter or condensations of an electromagnetic field.[1] This is secondary to the formation of a gravity wave or elastic deformation of a scaler potential existing outside of matter that does, however, maintain the configuration of solid bodies as well as space.[1] The gravity wave returns as a piezoelectric effect to produce a measurable disturbance of space-time itself, which results in a quantitized vibration of the atomic crystal lattice structure of gravitational or inertial masses contained by the biological system.[1,2] Genes, proteins, collagen, keratin, bone, cytoskeletal structures, and centrioles are piezoelectric structures, which convert electromagnetic oscillations to mechanical vibration (and vice versa). The PTEF operates as a force that is also manifested by vibrational waves, which can pass through plasma membranes. The permitivity of tissues change as the structure is vibrated by a gravity wave.[1,2]

Treatments that use electrical stimulation and magnetic fields have long been a topic of controversy and debate. During the last several decades, electromagnetic fields have been used for treatment of nerve regeneration, wound healing, graft healing, diabetes mellitus, myocardial ischemia, and cerebral ischemia.[3] Potential benefits of magnetic field therapies have included osteogenesis for the healing of delayed union and nonunion fractures as well as pseudoarthroses[4,5]; the control of malignant growths, healing of suppurative wounds associated with diabetes, size reduction of decubital ulcers, and treatment of varicose veins in humans have also been reported.[3,6-11] In rat models, results of healing of skin wounds that were exposed to low and extremely low pulsed electromagnetic fields varied.[3,4,12] Microbial growth has been inhibited after treatment with alternating low-frequency fields.[13] Horses with some musculoskeletal injuries have improved after receiving magnetic field therapy.[14]

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Clinical, experimental, and epidemiologic evidence supports the use of PTEF in the medical field.[15-29] The effects of PTEF on nerve regeneration after injury and cardiac innervation and arrhythmia formation have been investigated.[22-28] Empirical data suggest a beneficial effect of its use on joint pain, tendon and muscle injury, and osteoarthritis.[19,20] A specific effect of PTEF on skin healing has not been documented, and the mechanisms through which it may enhance wound healing are unproven.

The purpose of this study is to evaluate the effects of a low-intensity, extremely-low-frequency (pT) electromagnetic field on the healing characteristics of sutured and open skin wounds in rats. Tensile strength of sutured wounds, rate of contraction in open wounds, and extent of perfusion in both types of wounds were measured. The effects of PTEF on clinicopathologic, histologic, and microbiologic variables in rats were also assessed.

Materials and Methods

Animals—Sixty-four immature male Fischer-344 rats (weighing 350 to 430 g) were included in the study. Sixteen rats were randomly allocated to 1 of 4 groups. In 2 groups, rats with sutured wounds received either no treatment (sutured-control) or PTEF treatment (sutured-PTEF); in 2 groups, rats with open wounds received either no treatment (open-control) or PTEF treatment (open-PTEF). Four rats were assigned to each of the 8 treatment (control or PTEF) and day combinations (day 2, 4, 7, and 14).

Procedures—Procedures used in the study reported here were approved by the Institutional Animal Care and Use Committee at Mississippi State University. Prior to start of the study, rats were individually housed and allowed to acclimate to their surroundings for 7 days. Physical examinations were performed the day before surgery. On the day of surgery, each rat was anesthetized with ketamine[a] (80 mg/kg) and xylazine[b] (5 mg/kg) administered IP. The rats were positioned in sternal recumbency, and the area of the dorsal midline extending from the ears to the thoracolumbar region was clipped and prepared for surgery. Assessment of normal skin perfusion in the clipped area was determined in 10 randomly chosen rats by means of a laser Doppler perfusion imager[c] and associated computer software.[d] Each rat was positioned in sternal recumbency 35 cm beneath the laser Doppler imager, and measurements were obtained from the surgical site and surrounding normal skin. A consistent scan area was used for all rats. A scanning rate of 4 ms/pixel was used to allow a computation of the percentage (on a per-pixel basis) of reflected beam from moving blood cells. Some of the backscattered light was processed to obtain values for yield flux (proportional to blood flow) and the concentration of moving blood cells.

In 32 rats, with aseptic technique, a 3-cm linear incision was created along the dorsal midline over the cranial thoracic area. The incision was made in a cranial to caudal direction, and a template was used to ensure all incisions were of equal length. The incisions were then sutured with 4-0 nylon suture[e] in a simple continuous pattern. The sutures were equally spaced and placed through the dermis and epidermis. Skin edges were apposed, and the incision was closed with minimal tension. Laser Doppler perfusion imaging was performed on the surgical site immediately after surgery.

In the other 32 rats, a 1-cm × 1-cm full-thickness skin wound was created on the dorsal midline over the cranial thoracic area; a template was used to ensure all wounds were the same size. After surgery, the open wounds were photographed with a digital camera.[f] The camera was set on a fixed camera stand 52 cm above the rat. The images were recorded and evaluated with a digitizing software program[g] to determine total wound area. Laser Doppler perfusion imaging was performed on the surgical site immediately after surgery. All rats were returned to their cages in which a low-dust bedding material was used. No antimicrobials were administered, and no bandages were applied. Analgesia was provided by administration of liquid acetaminophen[h] (2 mg/mL in the drinking water) and butorphanol[i] (2 mg/kg, SC, q 12 h) for the first 5 days. All rats were observed daily to assess health and wound healing.

Starting on the day after surgery (day 1), each rat was placed within the PTEF unit[j] once daily. The rats were placed in special holding cages, allowing them to move about freely but keeping them separate from each other. The rats were acclimated to the holding cages 2 days prior to the day of surgery. Sixteen rats with sutured wounds and 16 rats with open wounds served as controls; they were placed in the PTEF unit for 160 minutes each day, but the unit was not activated. The remaining 32 rats (16 with sutured wounds and 16 with open wounds) were placed in the PTEF unit once daily.

Electromagnetic field therapy—The PTEF used in this study was generated by three 10-foot-diameter coils (constructed of 30-gauge copper wire) connected in series (Helmholtz) configuration.[j] The coils were driven by a frequency and amplitude adjustable sinusoidal precision generator and connected in series to an attenuator to obtain the preselected pT range field in the space between the coils. The unit was set initially at 271 Hz (frequency) and 9.69 mV (amplitude). This amplitude was defined as the amplitude from the generator, which was attenuated at 10^{-6} Gauss and transmitted to the coils. The frequency and amplitude of the electromagnetic field were gradually decreased every 10 minutes (individual test settings) for a total exposure time of 160 minutes. A pilot study was performed to ascertain the relative importance of amplitude, frequency, and duration settings for the PTEF unit. Eleven rats from a separate pilot study were used to estimate the main effects of the 3 setting factors and their 2-way interactions with resolution V full factorial design with 3 center points. A resolution V design is a design in which all main effects and 2-factor interactions can be estimated. Twelve treatment protocols were evaluated to determine the variables that positively affect wound healing. The rats were anesthetized, and a 3-cm linear incision was created in similar fashion to that performed for the sutured wound study. The rats were treated on a daily basis with the PTEF unit for 7 days. Wound healing was assessed by means of laser Doppler imager and tensiometry. Settings for the electromagnetic field unit were selected on the basis of data collected from the pilot study and data obtained from mouse studies.[26,27]

Assessment of Wound Healing

Sutured wounds—On days 2, 4, 7, and 14, 8 (4 control and 4 treated) rats with sutured wounds underwent evaluation. The rats were anesthetized with ketamine and xylazine as described. Laser Doppler perfusion imaging was performed as described to assess blood flow to the surgical site. Data were collected with the imaging software.

The percentage increase or decrease in yield flux and concentration at the wound site was determined by comparison with values obtained from normal tissue in the same area and from the original wound samples (collected before and immediately after surgery). A greater quantity of moving blood cells indicated a higher tissue perfusion; increased tissue perfusion indicates an increased inflammatory response at the surgical site.[29]

Blood samples (2 mL) were collected from each rat by cardiac puncture. A CBC and serum biochemical analyses were performed. Eight rats were euthanatized by barbiturate[k] overdose (0.5 mL, IP) and positioned in sternal recumbency.

A 6-cm × 10-cm rectangular section of skin that included the entire incision site was excised. Subcutaneous tissues were carefully removed from the skin sample with forceps, scissors, and a scalpel blade. A custom-manufactured solid aluminum template pound (cutting device) was set on the skin sample and struck with a rubber mallet to remove an identical- sized piece of skin from each sample. The excised piece of skin included the central 2 cm of the incision and a 2.5-cm flap of skin on each side of the incision; it was used for tensiometric testing. Each skin sample was wrapped in a sterile gauze sponge (moistened with sterile physiologic saline [0.9% NaCl] solution) and taken immediately to the tensiometer[l] for testing. The excess tissue (beyond the 2.5-cm flaps of skin on each side of the incision) was affixed in the tensiometer grips by use of 80-grit sandpaper to prevent slippage. New sections of sandpaper were used for each specimen tested. The initial length of the skin sample was determined as the grip-to-grip distance, which was measured after the initial application of a 0.7 N tensile force. The same grip-to-grip length of 5 cm was used for all samples. Samples were then stretched to failure in uniaxial tension at a rate of 5 mm/min. Force and displacement data were sampled at 1 Hz. Strain was calculated as the displacement divided by initial length. Cross-sectional area was recorded as the measured width of the sample multiplied by 2 mm. Stress was calculated as the force divided by cross-sectional area. Failure stress (strength [kPa]) was defined as the maximum stress reached before a decrease in force was first detected. The corresponding strain (expressed as a percentage) was defined as elongation at failure. Strain energy (toughness [mJ]) was defined as the area under the force-displacement curve (work done = force × displacement) up to the point of failure. The modulus of elasticity (stiffness [kPa]) was calculated as the slope of the linear portion of the stress-strain curve. A straight line was overlaid on the curve to indicate the range of data from which the modulus was calculated.

The cranial 0.5-cm portion of the incision that was not used for tensiometric testing was placed in neutral-buffered 10% formalin and prepared for histologic evaluation. The caudal 0.5-cm portion of the incision that was not used for tensiometric testing was removed by means of aseptic technique and placed in sterile physiologic saline solution for bacteriologic analysis.

Open wounds—On days 2, 4, 7, and 14, 8 (4 control and 4 treated) rats with open wounds were evaluated. The rats were anesthetized with ketamine and xylazine as described. Laser Doppler perfusion imaging was performed as described to collect perfusion data on the skin wound over the dorsal cranial thoracic region. The wounds were then photographed with the digital camera for planimetry analysis. Camera settings and positioning were identical to those used on day 0. The images were recorded and evaluated by means of a digitizing software program to determine total wound area and assess wound contraction. The percentage in reduction of total wound size resulting from contraction was determined by comparing the wound to the original wound as measured on day 0. Blood samples (2 mL) were collected from each rat by cardiac puncture. A CBC and serum biochemical analyses were performed. The rats were euthanatized by barbiturate overdose. A 2-mm × 2-mm biopsy specimen was excised from 1 corner of each wound by means of aseptic technique and placed in sterile physiologic saline solution for bacteriologic analysis. The remainder of the wound was placed in neutral-buffered 10% formalin for histologic examination.

Histologic examination—After fixation, skin samples were cut and imbedded in paraffin, sectioned at a thickness of 5 m, stained with H&E stain, and examined via light microscopy. Each sample was evaluated separately by a pathologist (RRP) who did not have prior knowledge of the treatment each sample had received. The inflammatory stage of healing was evaluated by means of 5 variables: numbers of neutrophils, mononuclear cells, plasma cells, and extent of edema and necrosis. Cell numbers were scored on a scale of 0 to 5 (0 = none, 1 = minimal, 2 = minimal to moderate, 3 = moderate, 4 = moderate to numerous, 5 = numerous). Edema and necrosis were scored on a scale of 0 to 5 (0 = none, 1 = mild, 2 = mild to moderate, 3 = moderate, 4 = moderate to severe, 5 = severe). The repair stage of healing was evaluated by means of 5 variables: number of fibroblasts, extent of neovascularization, collagen density, development of granulation tissue, and extent of epithelialization. Fibroblast numbers were scored on a scale of 0 to 5 (0 = none, 1 = minimal, 2 = minimal to moderate, 3 = moderate, 4 = moderate to numerous, 5 = numerous). Neovascularization was scored on a scale of 0 to 5 (0 = none, 1 = minimal, 2 = minimal to moderate, 3 = moderate, 4 = moderate to marked, 5 = marked). Collagen density was determined on the basis of the affinity of collagen fibers for eosin stain (0 = none, 1 = minimal, 2 = minimal to moderate, 3 = moderate, 4 = moderate to marked, 5 = marked). Granulation tissue was scored on a scale of 0 to 5 (0 = normal, mature, 1 = near maturity, 2 = intermediate, 3 = early to intermediate, 4 = some early appearance, 5 = immature). Epithelialization was also scored on a scale of 0 to 5 (0 = completely healed, intact epithelium, 1 = nearly intact epithelium, 2 = moderate amount of epithelium, 3 = moderate to minimal amount of epithelium, 4 = minimal amount of epithelial tissue, 5 = no intact epithelial tissue).

Bacteriologic evaluation—Samples were collected in an aseptic manner, although the wound sites were not cleansed with any surgical preparation scrub or solution. Skin biopsy specimens were homogenized in 1 mL of sterile physiologic saline solution; an aliquot of the homogenate was plated on 5% sheep blood agar, McConkey agar, and colistin-nalidixic agar.[m] The remainder of the homogenate was cultured in 5 mL of brain-heart infusion broth.[n] All cultures were incubated at 37°C in a 5% carbon dioxide atmosphere for up to 72 hours. Bacterial growths were identified via routine biochemical methods by the Diagnostic Laboratory Services at the College of Veterinary Medicine, Mississippi State University.

Clinicopathologic examination—The CBC included WBC and RBC concentrations, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, manual differential count, nucleated RBC count, platelet count, RBC morphology, and plasma protein estimation. The serum biochemical analyses included measurement of concentrations of glucose, BUN, creatinine, sodium, potassium, chloride, calcium, phosphorus, bilirubin, total carbon dioxide, total protein, albumin, cholesterol, and globulin; activities of alanine transferase and alkaline phosphatase; and estimation of anion gap, osmolality, and A/G ratio. Values were compared with known reference ranges for immature rats.

Statistical analyses—As reported by Scardino et al,[29] the median values and quantile deviations calculated at 10 days after treatment with a pulsed electromagnetic field on dogs were used to estimate the sample size needed for the study reported here. For each treatment group, the quantile deviation was converted to an SD value by assuming a normal distribution; the mean of the 2 SDs for each day was calculated to obtain an estimate of the common SD values for that day. The required sample size was computed for an 8-group (2 treatment groups measured on days 2, 4, 7, and 14) 1-way ANOVA.[30] Because our data were to be analyzed by use of the Kruskal-Wallis test, these sample size estimates were increased by a factor of $\pi/3$.[31] These calculations indicated that inclusion of 4 rats in each group at each day (64 rats

total) would provide power of at least 70% power to detect a difference between the treated and control groups.

Analyses of data initially included histographic and normal probability plot evaluations to determine the validity of assumptions needed for inferential statistical procedures. It was expected from other research findings[29] that the measures of healing used in this study would be skewed and requires either transformation or analysis by nonparametric procedures. The Kruskal-Wallis rank-sum test was used for analysis of nonparametric procedures to compare the 8 groups (2 treatment groups measured on days 2, 4, 7, and 14) or 10 groups (2 treatment groups measured on days 0, 2, 4, 7, 14) for each indicator of healing. If significant differences among groups were found, means were further separated by the least significant difference test after applying a rank transformation to the data. The transformed data were analyzed by use of a 1-way ANOVA. To characterize the clinical importance of any differences between treatments, approximate 95% confidence intervals for those differences were constructed from the untransformed data. Results of bacteriologic data were analyzed by Fisher exact test to determine the association between control and resonated wounds and the presence or absence of each bacterial isolate. Statistical computations were performed with computer software[o]; values of $P \leq 0.05$ were considered significant.

Results

Sutured wounds

During the study, anesthetic complications resulted in the deaths of 5 rats from the control groups (1 each from days 2, 4, and 14; 2 from day 7). No rats were removed from the PTEF-treated groups.

Tensiometric analysis—Initial pilot studies provided stress-strain plots for normal rat skin. In all normal rat skin samples tested, point of failure occurred when the tissues were loosened from the grips. In the rats of the study reported here, sutured skin failed at the incision site. The stress (strength), strain energy (toughness), and modulus of elasticity (stiffness) increased significantly in both PTEF-treated and control groups during the 14-day study **(Fig 1 and 2)**. Sutured wounds exposed to PTEF were significantly stronger (ultimate stress; $P = 0.018$) and tougher (absorbed energy; $P = 0.003$) than control wounds at day 14. Stiffness was not significantly different between PTEF-treated wounds and control wounds at any time period.

Laser Doppler perfusion imaging—In both PTEF-exposed and control rats, perfusion at the incision site was increased at day 2, compared with preoperative values. In the PTEF-treated wounds, perfusion decreased significantly ($P < 0.001$) at day 4 but increased significantly ($P = 0.009$) at day 7. No significant differences were found between PTEF-treated and control wounds in the percentage change in blood flow or the absolute increase in blood flow at any of the time periods investigated **(Fig 3)**. At day 14, perfusion levels were similar to those of the normal skin evaluated prior to surgery.

Histologic evaluation—On the basis of scores for histologic variables, variations in healing were observed between PTEF-treated and control wounds. Between these 2 groups, no significant differences were observed for numbers of neutrophils or mononuclear cells or for the extent of edema; plasma cells and necrosis were not detected in any of the samples. Fibroblast numbers were greater in PTEF-treated samples than in control samples ($P = 0.003$) at day 7 but were not significantly different at day 14. Neovascularization was greater ($P = 0.009$) in PTEF-treated samples than in control samples at day 7; however, neovascularization was greater ($P < 0.001$) in the control samples at day 14. Collagen density increased significantly ($P < 0.001$) in PTEF-treated and control samples between days 7 and 14 but was greater ($P = 0.048$) in PTEF-treated samples than in controls at day 14 **(Fig 4)**. Granulation tissue matured significantly ($P < 0.001$) in PTEF-treated samples and control samples between days 7 and 14 but was significantly ($P = 0.019$) more mature in PTEF-treated samples than in controls at day 7. Epithelialization was significantly greater in PTEF-treated samples than in controls at day 4 and 7 ($P = 0.017$ and 0.014, respectively).

Bacteriologic evaluation—Results of bacteriologic cultures indicated no significant differences between the bacterial content of PTEF-treated and control wounds. Nine different microorganisms were isolated. Common isolates included *Proteus mirabilis*, *Escherichia coli*, and *Enterococcus faecalis*; other organisms identified were *Staphylococcus epidermidis*,

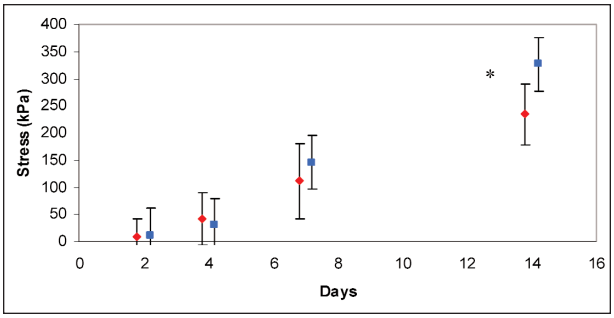

Figure 1—Graph of the ultimate stress or strength of sutured wounds in 16 pico-tesla electromagnetic field (PTEF)-treated (squares) and 11 control (diamonds) rats, as determined by tensiometry. Number of rats in each group examined at each data point: day 2, control (n = 3), treated (4); day 4, control (3), treated (4); day 7, control (2), treated (4); day 14, control (3), treated (4). Error bars represent 95% confidence intervals for the mean values. *Significant ($P < 0.05$) difference in wound strength in both groups observed on day 14, compared with values recorded on day 7.

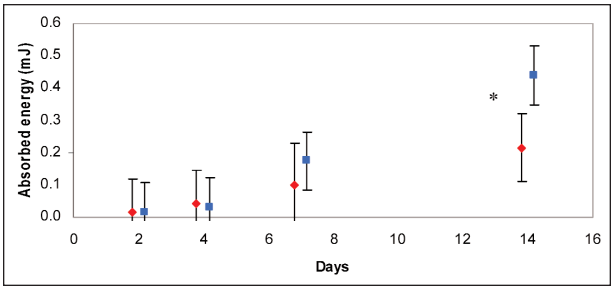

Figure 2—Graph of the strain energy or toughness of the sutured wounds in 16 PTEF-treated (squares) and 11 control (diamonds) rats. Number of rats in each group examined at each data point: day 2, control (n = 3), treated (4); day 4, control (3), treated (4); day 7, control (2), treated (4); day 14, control (3), treated (4). Error bars represent 95% confidence intervals for the mean values. *Significant ($P = 0.003$) difference in wound toughness in both groups observed on day 14, compared with values recorded on day 7.

Corynebacterium spp, *S aureus*, *Bacillus* spp, other *Staphylococcus* spp, and *Bacillus cereus*; *S aureus* was isolated only in the control group.

Clinicopathologic analysis—Results of CBCs and serum biochemical analyses were not significantly different between PTEF-treated and control rats at any of the time periods; values were within published reference values for rats of this age.

Open wounds

During the study, anesthetic complications resulted in the deaths of 5 rats from the control groups (1 each from days 2, 4, and 14; 2 from day 7). No rats were lost from the PTEF-treated groups.

Laser Doppler perfusion imaging—Perfusion significantly increased immediately after surgery but decreased significantly at day 2 in both PTEF-treated and control wounds. In both groups, perfusion immediately after surgery and at day 2 was significantly higher than values recorded before surgery; perfusion returned to baseline (presurgical) values by day 14. No significant differences in perfusion were observed between PTEF-treated and control wounds at any time period. Laser Doppler values for the wounds were compared to values obtained before and immediately after surgery to allow consistency in the measurements.

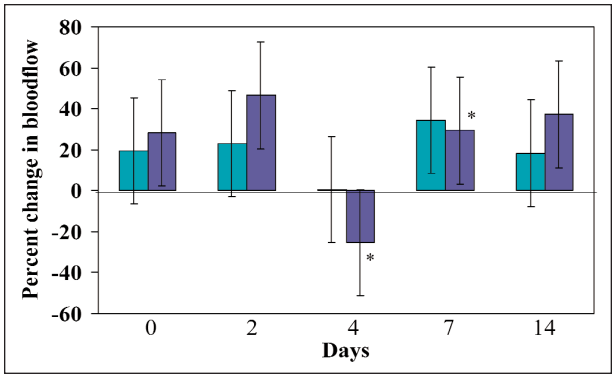

Figure 3—Graph of the results of laser Doppler perfusion imaging of the sutured wounds in 16 PTEF-treated (dark bars) and 11 control (light bars) rats. Number of rats in each group examined at each data point: day 2, control (n = 3), treated (4); day 4, control (3), treated (4); day 7, control (2), treated (4); day 14, control (3), treated (4). Error bars represent 95% confidence intervals for the mean values. *Significant ($P < 0.001$) decrease in perfusion on day 4 in the PTEF-treated group, compared with perfusion recorded on day 2. Perfusion in the treated group increased significantly ($P < 0.001$) on day 7, compared with the value recorded on day 4. Perfusion values were compared to normal values from data collected prior to surgery.

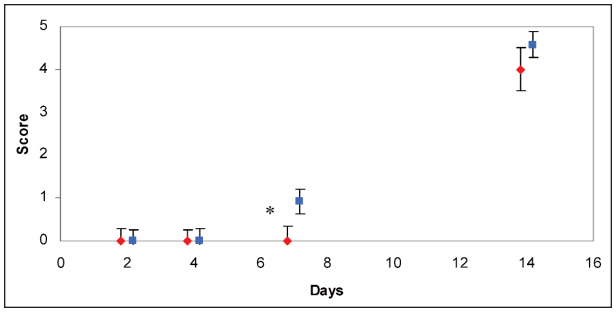

Figure 4—Graph showing the results of collagen density for PTEF-treated sutured wounds in 15 PTEF-treated (squares) and 12 control (diamonds) rats. Number of rats in each group examined at each data point: day 2, control (n = 3), treated (4); day 4, control (4), treated (3); day 7, control (2), treated (4); day 14, control (3), treated (4). Error bars represent 95% confidence intervals for the mean values. *Significant ($P = 0.048$) difference between groups (days 7 and 14).

Planimetric evaluation—Results of planimetric evaluation of the digital images from each wound revealed a significant ($P < 0.001$) decrease in wound size in PTEF-treated wounds at days 2 and 4, compared with control wounds (**Fig 5**). All wounds in both groups were significantly decreased in size at days 7 and 14, compared to day 0.

Histologic evaluation—On the basis of scores for histologic variables, variations in healing were observed between PTEF-treated and control wounds. Neutrophil numbers were significantly ($P < 0.001$) lower in PTEF-treated samples than in controls at day 14. Mononuclear cell numbers were significantly lower in PTEF-treated samples at days 2 and 14 ($P = 0.003$ and < 0.001, respectively). Plasma cells and necrosis were not observed in any of the samples. Edema was

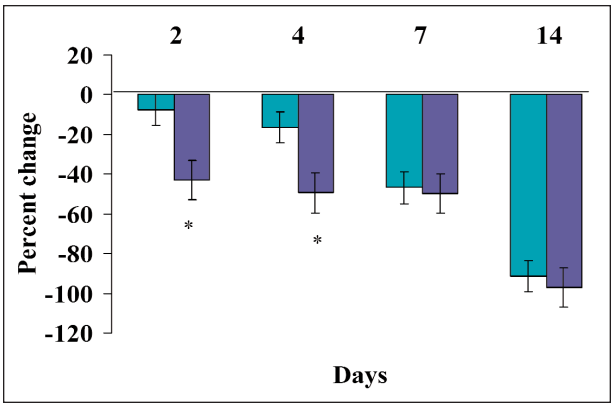

Figure 5—Graph of the total wound surface area with percentage change during the wound healing study in 16 PTEF-treated (dark bars) and 11 control (light bars) rats. Number of rats in each group examined at each data point: day 2, control (n = 3), treated (4); day 4, control (3), treated (4); day 7, control (2), treated (4); day 14, control (3), treated (4). Error bars represent 95% confidence intervals for the mean values. *A significant ($P < 0.001$) difference between groups (days 2 and 4).

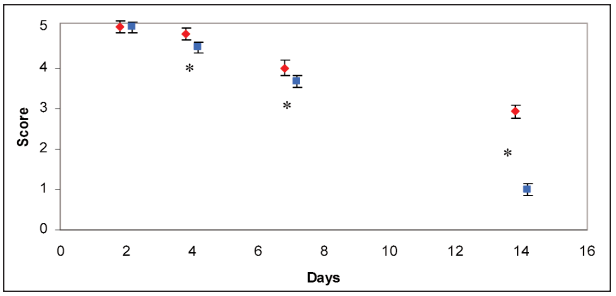

Figure 6—Graph of epithelial tissue scores for open wounds in 16 PTEF-treated (squares) and 11 control (diamonds) rats. Number of rats in each group examined at each data point: day 2, control (n = 3), treated (4); day 4, control (4), treated (4); day 7, control (2), treated (4); day 14, control (3), treated (4). Error bars represent 95% confidence intervals for the mean values. *Significant difference between groups (days 4, 7, and 14; $P = 0.002$, 0.008, and < 0.001, respectively).

significantly less severe in PTEF-treated samples than in controls at days 2 and 14 ($P = 0.034$ and 0.01, respectively). Fibroblast numbers were significantly ($P < 0.001$) increased in both groups between days 7 and 14. At day 14, the numbers of fibroblasts were greater ($P < 0.001$) in the PTEF-treated rats than in the control group. Neovascularization significantly ($P < 0.001$) decreased in both groups between days 4 and 14. Collagen density increased significantly ($P < 0.001$) in both groups between days 7 and 14 but was significantly ($P < 0.001$) increased in PTEF-treated samples at days 4 and 14. Granulation tissue matured significantly ($P < 0.001$) in both groups between days 2 and 14, and it was significantly ($P < 0.001$) more mature in PTEF-treated samples than controls at day 14. Epithelialization improved ($P < 0.001$) in PTEF-treated and control samples between days 4 and 7 and between days 7 and 14. Epithelialization was significantly better in the PTEF-treated than in controls at days 4, 7, and 14 ($P = 0.002$, 0.008, and < 0.001, respectively; **Fig 6**).

Bacteriologic evaluation—Results of bacteriologic cultures indicated no significant differences between the bacterial content in PTEF-treated and control wounds (**Fig 7**). Eleven different microorganisms were isolated. Common isolates included *P mirabilis*, *E coli*, and *E faecalis*. Other organisms identified were *S epidermidis*, *Corynebacterium* spp, *S intermedius*, *Acinetobacter iwoffi*, *S aureus*, *Bacillus* spp, other *Staphylococcus* spp, and *B cereus*. No growth of *A iwoffi* or *B cereus* was detected in the control group. Two of the microorganisms (*S intermedius* and *A iwoffi)* were isolated from the open wounds but not from wounds in the sutured group.

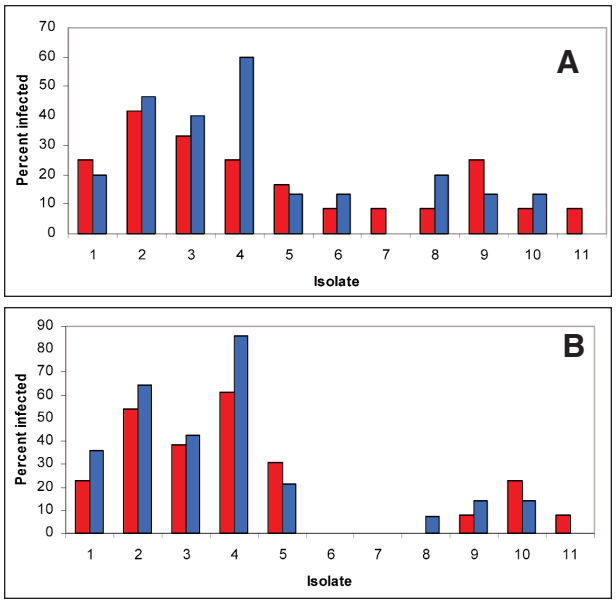

Figure 7—Percentage of sutured (A) and open wounds (B) infected by bacterial isolates (identified by culture) in 14 (sutured) and 15 (open) PTEF-treated (dark bars) and 13 (sutured) and 12 (open) control (light bars) rats. Wounds were examined at days 2 (control [n = 3], treated [4]), 4 (control [3], treated [4]), 7 (control [2], treated [4]), and 14 (control [3], treated [4]). Note that *Staphylococcus intermedius* and *Acinetobacter iwoffi* were not isolated in the sutured wounds. Isolate 1 = *S epidermidis*. Isolate 2 = *Proteus mirabilis*. Isolate 3 = *Escherichia coli*. Isolate 4 = *Enterococcus faecalis*. Isolate 5 = *Corynebacterium* spp. Isolate 6 = *S intermedius*. Isolate 7 = *A iwoffi*. Isolate 8 = *S aureus*. Isolate 9 = *Bacillus* spp. Isolate 10 = *Staphylococcus* spp. Isolate 11 = *Bacillus cereus*.

Clinicopathologic analysis—Results of CBCs and serum biochemical analyses were not significantly different between PTEF-treated and control rats at any of the time periods; values were within reference ranges for rats of this age.

Discussion

The potential benefits of electromagnetic field treatment on biological tissues have been documented in both human and veterinary medical literature.[4-12,19-29,32-50] Static and dynamic electromagnetic fields have been studied. Static magnetic fields have a constant electric voltage and include electromagnets with a constant DC charge or fields created by a ferrous magnet. Dynamic magnetic fields are variable energy fields that are continuously changing. They include pulsating electromagnetic fields, low-intensity pulsed ultrasound, sinusoidal electromagnetic fields, and PTEFs like that used in the study reported here.[9] Currently, most of the magnetic fields used for medical therapy and research are dynamic magnetic fields with a changing electrical voltage. Dynamic fields have a greater effect on wound healing in rats than do static fields.[34] Our study evaluated the effects of a low-intensity, extremely-low-frequency (pT) electromagnetic field on sutured and open skin wounds in rats. To the authors' knowledge, no studies have been performed to investigate the effects of PTEF on healing of skin wounds with assessment of clinicopathologic and histologic variables and bacteriologic growth characteristics at the wound sites.

Tensiometric testing was used to evaluate the material properties of the sutured skin wounds, including their stiffness, strength, and toughness. Stiffness is a measure of the incision's distensibility and was determined by the modulus of elasticity (slope of the stress vs strain curve in the elastic region). Strength is defined as a tissue's ultimate stress or the maximum attainable stress beyond which the tissue fails or an increase in strain occurs without an increase in stress. The maximum stress required to disrupt the incision determined its strength. Toughness is defined as the area under the force versus deflection curve and was a measure of how much energy was absorbed by the incision until the moment of rupture.

At day 14, the strength and toughness of the sutured wounds exposed to the PTEF were significantly greater than control wounds. This may be attributable to an increase in collagen formation and maturation within the wounds. Collagen contributes extensively to the tensile strength and toughness of wounds and is deposited by fibroblasts within the granulation tissue during the repair phase of wound healing. Granulation tissue was more mature in PTEF-treated incisions; at day 7, the number of fibroblasts in PTEF-treated incisions was increased, compared with the number in controls. A significant increase in collagen density was identified histologically in the PTEF-treat-

ed and control incisions between days 7 and 14; collagen density was greater in PTEF-treated incisions than that of control incisions at day 14. Collagen density was subjectively determined by assessment of the affinity of fibers for eosin stain.

Collagen fibers are initially oriented randomly in a wound. During the maturation phase of wound healing, strength increases as the collagen matures; crosslinking increases, and the orientation of fibers becomes more organized. Eventually, the well-oriented collagen fibers form bundles that become increasingly difficult to distinguish from the surrounding dermis at the wound edges. Chvapil et al[51] observed that the maximal rate of collagen synthesis is reached on day 10 in rats. This is consistent with our finding of increased collagen density in both PTEF-exposed and control incisions between days 7 and 14 and with the increased strength and toughness of the incisions at day 14. A correlation has also been described[41,51] between hydroxyproline concentration (a measure of collagen concentration) and the breaking strength of tissues. Hydroxyproline concentration increases rapidly by the fourth day after injury and reaches highest values between 5 and 14 days[41]; although hydroxyproline concentration was not specifically measured in our study, high concentration at day 14 would not be unexpected because of increased collagen density, wound strength, and toughness detected.

In addition to increased strength, incisions in the PTEF-treated group in general also exhibited significantly greater stretch to failure (extensibility). It is suspected that the increased extensibility resulted from changes in elastin or collagen density or tissue organization. The combination of greater strength and extensibility provided increased toughness (energy absorbed to failure) in the PTEF-treated group, compared with that of controls.

At days 2 and 4, open wounds that were exposed to the PTEF were significantly smaller than control wounds. This reduction in the total wound area was similar to a finding reported by Scardino et al[29]; in that study, wounds were treated with a pulsed electromagnetic field. Other investigators have reported[52-54] rapid reduction in wound size after application of various topical treatments. The early reduction in wound size may be attributable to changes that develop during the initial inflammatory phase of wound healing, which lasts approximately 3 to 5 days.[55] Immediately after full-thickness skin loss, hemorrhage occurs, edema develops, and small blood vessels constrict. A clot forms, followed by development of a scab if the wound remains undisturbed. Cells, predominately leukocytes, move into the injured area during this phase of inflammation. Electromagnetic fields are reported[56] to affect proliferation of inflammatory lymphocytes. In our study, numbers of neutrophils (day 14) and mononuclear cells (days 2 and 14) differed between rats with PTEF-treated wounds and those with untreated wounds. At day 2, there was significantly less edema observed in the PTEF-treated open wounds and adjacent tissues than control wounds. Less edema may have reduced swelling and tension in the tissues, allowing the wounds to contract more quickly. The greater extent of edema and swelling in the control open wounds may have increased tension around the wound, which caused the skin to pull away from the wound edges, thereby making the control wounds comparatively larger.

Cells associated with inflammation produce a variety of cytokines, the cellular regulators of inflammation that are typically released on the third or fourth day after injury.[57] Specific cytokines, including **transforming growth factor-beta** (**TGF-β**), have been evaluated in wound healing studies[56,58] involving rats. It appears that TGF-β promotes differentiation of fibroblasts into myofibroblasts and can stimulate contraction in vitro; TGF-β is the only cytokine produced at high levels in rats.[59] Whether elevated concentration of TGF-β could explain the increased rate of wound contracture in PTEF-treated wounds in our study remains to be elucidated.

The repair stage of wound healing consists of fibroblast migration, capillary infiltration, and epithelial proliferation and migration.[60,61] Fibroblast response and proliferation at a wound site are affected by several factors, including the effects of inflammatory products, chemotactic factors, and growth factors produced by platelets, lymphocytes, and macrophages. Migration of fibroblasts on fibrin networks and their infiltration of capillaries (usually observed at 3 days after injury) are important in the formation of granulation tissue in the wound. In 1 study[62] of open wounds in rats, contraction was caused by fibroblasts, and myofibroblasts were not required. Granulation tissue is important in wound contracture, which typically begins approximately 3 to 6 days after creation of the wound.[55,57] In the sutured wounds investigated in our study at day 7, fibroblast numbers were greater, and granulation tissue was significantly more mature in PTEF-treated wounds than that of control wounds. In open wounds investigated in our study at day 14, granulation tissue was more mature in wounds in the treated group than in those of the control group. Evidence of granulation tissue formation in open wounds was initially observed on day 4 and may have contributed somewhat to early wound contraction. The PTEF appeared to have a positive effect on the maturation of granulation tissue in sutured and open wounds.

Wound epithelialization occurs by proliferation of epithelial cells from the hair follicles and associated glands as well as migration of epithelial cells from the epidermis at the skin edge.[63] Rats do not have sweat glands; therefore, the follicles, sebaceous glands, and dermal structures (including collagenase and reticular fibers, fibroblasts, and fibronectin) are responsible for epithelial tissue formation by forming a framework for migration of epithelial cells. Early in the process of wound healing, epithelial tissue is formed along the wound edge; as granulation tissue is formed, a base for epithelial migration is established. Electromagnetic treatment has been shown to have a positive effect on epithelial migration, and in a study[29] of wound healing in dogs treated with a pulsed electromagnetic field, increased epithelial migration was observed at days 10 to 15. In the sutured wounds examined at days 4 and 7 in the study reported here, epithelialization was more

pronounced in PTEF-treated wounds than that observed in control wounds. In open wounds examined at days 4, 7, and 14, epithelialization was more pronounced in PTEF-treated wounds than that observed in control wounds.

Laser Doppler imaging was used to noninvasively monitor microvascular blood flow without affecting the general state of perfusion.[64] The imaging unit used an optical fiber to transmit a low-power laser light to the tissue, and the light was scattered by the moving blood cells in the microvasculature. Moving blood in the microvasculature caused a Doppler shift in the light, which was processed by a computer to build a color-coded image of blood flow.[64] Some of the backscattered light was processed to yield flux (proportional to blood flow) and concentration (proportional to the concentration of moving blood cells) readings. For skin, it is assumed that the full thickness of the dermis is probed by wavelengths of light in the red and infrared ranges. However, the infrared wavelength gives a higher weighting to blood flow in the deeper dermis. The depth of tissue probed by the imaging unit may also be affected by pigmentation and crust or scab formation. The albino rats used in this study did not have skin pigmentation to impede laser penetration. To the authors' knowledge, studies to evaluate laser penetration of open wounds have not been performed. Results of studies[65,66] involving burn depth assessment in humans indicated that the accuracy of laser Doppler imaging correlated with histologic findings in 100 and 96% of cases and burns, respectively. Those wounds included superficial and deep dermal burns with blisters and transparent eschars. In areas of skin with crusts and scabs, however, the manufacturer of the laser Doppler device reports that penetration is affected by the thickness of the material. In the investigation of open wounds in our study, scab formation may have reduced the laser Doppler measurements obtained; measurements were increased or normal when healing was near completion or finished. These findings were similar to those of another study.[29] In our study, the open wounds that were measured had been created along the dorsal midline. As the wound contracted and epithelialized during healing, the tissue type in which perfusion was being measured gradually changed. Initially, there was an open wound and little intact skin. Later, as healing progressed, the area of open wound was small, and the area of normal skin increased. However, an identical scan area was evaluated in control and PTEF-exposed wounds to account for this change in tissue type as the wounds healed. Also, perfusion data collected serially after wounding were compared to perfusion data obtained prior to and immediately after the open wound was created. Ultimately, data collected via the laser Doppler perfusion imager revealed no differences between control and PTEF-exposed wounds at any time period.

In a study by Ottani et al,[4] electromagnetic fields influenced new vessel growth and promoted the early development of a vascular network during wound healing.[4] At day 6 in that study, an increased rate of wound healing was apparent (associated with proliferation of reticular connective tissue with a high number of fibroblasts and the development of a new vascular network). In our study, however, there appeared to be little correlation between Doppler perfusion measurements and histologic evaluation of neovascularization, which may be a consequence of a difference in size and flow of the vessels imaged in the 2 studies.

Results of laser Doppler imaging obtained in our study were different than those reported[29] by Scardino et al; those investigators observed a progressive increase in perfusion in healing wounds in adult Beagles treated with a pulsed electromagnetic field from day 0, which peaked at day 3 to 5 and decreased by day 10. A similar pattern of perfusion was detected in the control rats of the study reported here, in which perfusion increased at day 2 and progressively decreased with time to preoperative values. Interestingly, perfusion of the sutured wounds exposed to PTEF increased at day 2, decreased at day 4 (as expected), and increased at day 7. This second increase at day 7 is unexplained and is not corroborated by histologic evidence of increasing neovascularization. However, perfusion (as measured with the laser Doppler device) was not significantly different between groups at any time period. Open wounds had marked increase in perfusion at day 0 (immediately after surgery), which was likely a result of the surgical procedure and exposure of the underlying musculature. Perfusion in those open wounds progressively decreased at days 2, 4, and 7, with a slight increase again at day 14. Comparisons of these data cannot be made with other studies involving rats as models, because laser Doppler imaging was not used.

In veterinary medicine, the combination of ketamine and xylazine is commonly used to achieve anesthesia. Ketamine is a dissociative anesthetic agent that produces anesthesia and analgesia. Xylazine is an α-2 adrenergic agonist with analgesic, sedative, and muscle-relaxant properties. Both agents have been shown to cause hypoventilation when used alone.[67] Ketamine, alone or in combination with xylazine, also causes hypercarbia, acidosis, and induces $\geq$ 10% depression in the arterial partial pressure of oxygen.[67] Xylazine has vasoconstrictive properties.[68,69] Perfusion to the skin can be affected by ketamine-xylazine administration because of a reduction in mean arterial blood pressure.[60] In the rats of the study reported here, blood pressure measurements were not monitored during the anesthetic episodes; however, the same anesthetic protocol was used for measurements of blood perfusion before and after surgery in PTEF-treated and control rats. Therefore, any affect of anesthesia on perfusion measurements should have been consistent among groups.

Ketamine-xylazine has long been considered a suitable anesthetic combination for use in laboratory animals. Compared with other agents, administration of ketamine-xylazine produces the greatest depth of anesthesia, and the combination is particularly appropriate for procedures that involve tissue manipulation. No deaths were reported in a study[67] that evaluated 3 different dosages of ketamine and xylazine in adult male rats, but high doses of xylazine can cause acute pulmonary edema in rats.[70] In the rats of our study, some deaths occurred; results of postmortem examina-

tions did not include detection of gross or microscopic changes, and the deaths were attributed to the anesthesia. Administration of a lower dose of ketamine-xylazine and improved monitoring during recovery from anesthesia may have reduced the number of deaths. Agents, such as α_2-adrenergic antagonists (yohimbine and tolazoline), can partially reverse the effects of xylazine. However, neither of the reversal agents was reported to improve the pronounced hypothermia associated with administration or produced by ketamine-xylazine in rats.[71]

Common bacterial isolates from normal rat skin include *S warnerii*, *S xylosus*, and *S epidermidis*.[72] Only *S epidermidis* was cultured from skin wounds in the rats in our study. It is unlikely that this difference was associated with PTEF treatment, because the type and numbers of bacteria isolated from PTEF-treated and control wounds in our study were not significantly different. *Staphyloccocus intermedius* and *A iwoffi* were isolated from open wounds but not from sutured wounds.

No significant differences were observed between clinicopathologic variables measured in PTEF-treated and control rats. Other investigators[72,73] have evaluated results of CBCs (specifically, neutrophil and lymphocyte concentrations), plasma fibrinogen concentration, serum lactate dehydrogenase activity, and blood glucose concentration as possible markers of wound infection in rats. Serum glucose concentration was not significantly different between PTEF-treated and control rats. Plasma fibrinogen concentration and serum lactate dehydrogenase activity were not evaluated in the study reported here.

Exposure to the PTEF caused no adverse effects on clinicopathologic, histologic, or bacteriologic variables tested in this study. From our data, it appears that PTEF is a safe form of adjuvant treatment for wounds and improves strength of sutured wounds and speeds contraction of open wounds. Whether variations in PTEF unit settings might improve the healing of complicated wounds, including decubital ulcers, burns, skin grafts, and skin infections, remains to be determined.

[a]Ketaset, Fort Dodge Animal Health, Fort Dodge, Iowa.
[b]Rompun, Bayer Corp Animal Health, Shawnee Mission, Kan.
[c]Moor laser Doppler perfusion imager, Moor Instruments Inc, Wilmington, Del.
[d]Moor LDI-2 software, Version 3.0 and version clinical 1.0, Moor Instruments Inc,Wilmington, Del.
[e]Ethilon, Ethicon Inc, Somerville, NJ.
[f]Nikon Cool Pics 995, Nikon Inc, Melville, NY.
[g]Oncor Image, Image analysis system, Version 2.0.5d, Oncor Inc, Gaithersburg, Md.
[h]Acetaminophen oral solution, Goldline Laboratories Inc, Miami, Fla.
[i]Torbugesic, Fort Dodge Animal Health, Fort Dodge, Iowa.
[j]Jacobson pico-tesla electromagnetic field therapy unit, Institute of Theoretical Physics and Advanced Studies for Biophysical Research, Jupiter, Fla.
[k]Beuthanasia-D, Schering-Plough Animal Health, Union, NJ.
[l]Instron model 1011, Instron Corp, Canton, Mass.
[m]Remel, Lenexa, Kan.
[n]Difco Laboratories, Detroit, Mich.
[o]SAS and SAS/STAT software, SAS Institute Inc, Cary, NC.
[p]Clayton L, Supervisor of Prototype Development Laboratories, Stennis Space Center, Stennis Space Center, Miss: Personal communication, June 2002.

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