We have recently demonstrated that some forms of cathodal high voltage pulsed current (HVPC) curb posttraumatic edema formation in frog hind limbs. The purpose of this study was to determine, by assessing the capacity of anodal HVPC to curb posttraumatic edema formation, whether polarity is an important variable. Fourteen anesthetized bullfrogs were placed on large dispersive electrodes lining body slings that maintained the frogs' limbs in a dependent position throughout data collection. The frogs' feet were traumatized by impact following initial measurement of limb volumes. At the commencement of each of four 30-minute treatments, hind limbs were immersed in separate beakers and briefly stimulated until motor threshold was determined. One limb, randomly selected, received anodal HVPC at 90% of motor threshold and 120 pulses per second; the other limb served as a control. Treatments were followed by 30-minute rests. Limb volumes were measured by water displacement immediately after trauma and following each treatment and rest period. Data were expressed as changes from pretrauma volumes in milliliters per kilogram of body weight. A repeated-measures analysis of variance was used to test for treatment effect. Despite an aggressive series of treatments, virtually symmetrical bilateral edema occurred; therefore, no treatment effect was evident. This result contrasts with treatment effects previously reported for cathodal HVPC. [Fish DR, Mendel FC, Schultz AM, Gottstein-Yerke LM. Effect of anodal high voltage pulsed current on edema formation in frog hind limbs. Phys Ther. 1991;71:724–733.]

Key Words: Edema, Electrical Stimulation, Electrotherapy, Inflammation, Trauma.

Electrical stimulation (ES) is commonly used for controlling edema and joint effusion in patients. High voltage pulsed current (HVPC) has been advocated as a means of reducing posttraumatic edema and joint effusion, but the efficacy of HVPC for these purposes is largely unknown. Griffin and colleagues recently provided the only published clinical evidence that suggests HVPC may reduce chronic posttraumatic hand edema, but conclusive evidence of HVPC's capacity to control edema in humans awaits further research.

A series of studies performed in our laboratory have shown that cathodal HVPC, applied at 120 pulses per second (pps) and at intensities 10% less than those needed to evoke visible muscle contractions, curbs posttraumatic edema formation in the hind...
The purpose of this study, therefore, cited, stimulation was applied with 30-minute treatment on posttraumatic was to assess the capacity of anodal edema formation. In that study, however, is not known. In each of the polarity, however, is not known. In studies using a rat model, paws are often injured after administration of anesthesia and limb volumes are determined repeatedly by plethysmography (ie, immersion of limb and measurement of displaced water). Small mammals, including rats, however, do not readily tolerate prolonged anesthesia. Their high metabolic rates preclude prolonged anesthesia without intravenous feeding. Heat and water loss are also difficult to counteract without tampering directly with their vascular systems.

To better control variables and reduce time and cost, nonhuman models, namely rats, have been used extensively to probe the effectiveness of various treatments, especially those involving anti-inflammatory medications, on posttraumatic swelling. In studies using a rat model, paws are often injured after administration of anesthesia and limb volumes are determined repeatedly by plethysmography (ie, immersion of limb and measurement of displaced water). Small mammals, including rats, however, do not readily tolerate prolonged anesthesia. Their high metabolic rates preclude prolonged anesthesia without intravenous feeding. Heat and water loss are also difficult to counteract without tampering directly with their vascular systems.

Such interventions may well affect activities at the capillary level. If anesthetized for trauma, then the animals must either be restrained after they wake, a very stressful situation for most animals, or allowed free movement, which may itself affect limb volumes by displacing fluids via muscle pumping. Free movement may also subject the animal to pain and the injured body part to further injury or manipulation (eg, massage, licking), which may affect limb volumes. Limb volumes must be determined on relaxed, perfectly still limbs (ie, on anesthetized animals), and treatment (eg, ES) is delivered more effectively and with less evoked stress when the animal is anesthetized. Repeated use of anesthetics within 1 day or even once a day, however, often results in death.

To avoid some of the problems associated with human subjects or small mammals, we have chosen bullfrogs as the experimental model for this and a series of related studies on edema. Bullfrogs can be anesthetized (through their skin) for an entire day without interdicting their vascular systems, and pain, stress, and movement ("muscle pumping") are eliminated as confounding factors. Repeated limb-volume measurements are easily accomplished, and ES treatments are administered without inducing stress or discomfort. Ordinary room temperature is certainly within tolerance of free-ranging bullfrogs and is probably near the high end of this amphibian's preferred range. Heat loss, therefore, is less a problem at room temperature for anesthetized poikilothermic (cold-blooded) frogs than for anesthetized homeothermic (warm-blooded) small mammals. Water loss is easier to control for amphibians than for small mammals because anesthetized amphibians breathe through their skin and therefore lose little moisture from their respiratory trees. Simply keeping amphibians moist effectively negates water loss.

Anesthesia, of amphibians or mammals, slows heart rate and probably most other physiologic responses, including responses to trauma. Observations of traumatized anesthetized animals (amphibians or mammals), however, indicate that fairly typical physiologic responses do occur, albeit at a rate that may be different from that of fully alert animals. Such observations also indicate that the physiologic responses for each class of vertebrate are similar in kind and in function and therefore that their physiologic processes are probably similar. Just as giant axons of squids have served to reveal many principles of mammalian neurophysiology and eggs of other invertebrates have served as a model for mammalian fertilization, so may experiments using the hind limbs of bullfrogs suggest the clinical utility of ES.

Our studies of the effects of various forms of ES on edema have been designed primarily to determine whether there is any treatment effect and, if so, why. Although ES is com-
monly applied to control edema, we have chosen not to model our study protocols after any published clinical method, in part, because no clinical protocol has been demonstrated to be effective. Indeed, if the influences of treatment variables such as polarity, stimulus amplitude, pulse duration, and frequency were already known and formed the bases of one or more protocols, there would be no purpose for our series of studies. Not only are present clinical protocols unsubstantiated, but such variables as duration of clinical treatments for controlling edema with ES seem arbitrary and based more on exigencies of patient scheduling than demonstrated optimal treatment effects.

Most clinical protocols using ES include the application of ice, elevation, and compression. Inclusion of any of these standard techniques in our protocols would make it impossible to determine the efficacy of ES alone. Some clinical protocols also suggest that stimulus amplitudes be increased to motor levels. In all but one of our studies, we have chosen to treat for a minimum of 2 hours (in four 30-minute blocks interspersed with rest periods of 30 or 60 minutes) and at an intensity of 90% of visible motor threshold. We chose an aggressive protocol so that, if ES worked, it would have ample time to do so, but we did not wish to invoke a "muscle pump" because we wanted to separate exercise effects from other direct effects of ES.

In all of our studies, we placed frogs in slings (Fig. 1) to simplify handling and to provide a means of uniformly supporting frogs during plethysmography, treatment, and rest in a way that rendered all four limbs pendant (ie, in a dependent position). Hind limbs were required to be in a dependent position at least for plethysmography and treatment, because hind limbs were immersed to determine volume displacement and for administration of treatment ("immersion technique"). In our earlier studies, frogs were placed prone during rest periods, but, in our more recent studies, frog limbs remained in a dependent position throughout data collection. Such positioning alone is known to increase limb volumes in anesthetized frogs. Therefore, it seems reasonable to expect dependent positioning to exacerbate post-traumatic edema formation.

Fig 1. System for measuring frog hind-limb volumes. (Reprinted with permission of the American Physical Therapy Association.)

In our model, both feet of each frog are traumatized equally so that net increases in limb volumes include swelling induced by trauma as well as positioning. Whereas dependent positioning is not used in conventional clinical practice, orienting the limbs of our frogs in virtually any other position might be construed as therapeutic. Efficacy of ES for edema control might be underestimated using our current model because dependent positioning may mask a small, but positive, treatment effect. Thus, when we report that ES results in significant treatment effects, such effects occur despite the challenge of dependent positioning. That is, we are now imposing a more stringent test of efficacy of ES to ensure that positioning is not a factor.

Another method common to our studies is the use of the "immersion technique." In this technique, water into which a limb is immersed serves as the distal electrode. This method is used clinically, but we selected it because it eliminates problems associated with surface electrodes (eg, uneven contact with skin, particularly on the small, irregularly shaped body parts of small vertebrates). This method also eliminates difficulties in
applying electrodes for long periods, especially under the conditions of our studies. We keep frogs moist and anesthetized by continuously dripping anesthetic-laden water over them during rest periods between treatments. Moreover, measuring limb volumes by plethysmography requires repeated immersions in water. This repeated exposure to moisture results in dissolution of electrode gel/adhesive. Reapplication of electrodes then requires frequent manipulation of injured limbs and potentially affects limb volumes.

In all studies in our series, we traumatized both feet of each animal using a uniform method (weight drop or hyperflexion). Frogs within a study varied in body weight, therefore, dropping the same weight from a uniform height did not inflict uniform injuries across the group. Smaller animals apparently received greater injuries than larger animals. We chose not to normalize impact forces relative to body weight because we believe such normalization is unnecessary when using each animal as its own control. Our design requires only the assumption that treated limbs as a group are injured to the same extent as untreated limbs. In our studies, each treated limb had a near-perfect untreated match, namely the contralateral limb. We therefore believe we met the requirements of our assumption. We also recognized that normalized impact forces would not produce uniform injuries in all animals because of variations in factors such as age, sex, nutritional and hormonal status, reproductive status, musculoskeletal development, inherent differences in development of vascular and lymphatic systems, and "efficiency" of the myriad systems responsible for wound healing. In our studies, both feet of each animal were traumatized as uniformly as possible and all treated limbs were compared statistically with all untreated limbs, which were handled in precisely the same way as treated limbs except for stimulation.

Our model, like any other, has limitations. Frogs and humans, as fellow vertebrates, share significant and fundamental physiologic characteristics. If they did not, then clearly the frog could not serve as a "model" for humans. Frogs have long been used as models for humans in physiologic studies similar to ours that have examined inflammatory responses and wound healing. Indeed, many basic tenets of electrotherapy have been determined on amphibians, frogs in particular, and applied to mammals with good fidelity. The physiology of frogs and that of humans also differ in significant ways so that results of studies of frogs cannot be applied directly to humans. We believe, however, that the physiology of humans and frogs is sufficiently close that results of studies on frogs are predictive of similar results in humans, particularly as a basis for the generation and initial clinical exploration of hypotheses.

Method

Subjects

Fourteen bullfrogs (Rana catesbeiana), weighing 288 to 519 g (X=403, SD=75), were used in this study. All methods relating to the care and handling of the animals were approved by the Institutional Laboratory Animal Care Committee at the State University of New York at Buffalo.

Instrumentation

Trauma was induced by impact as in some of our previous studies. A steel rod weighing 358 g was dropped through a vertical tube from a height of 66 cm. A rectangular piece of plastic (2×2×0.5 cm) was interposed between the hind limb and the tube to distribute the force of impact and prevent rupture of skin. Limb volumes were determined by a water-displacement apparatus consisting of an immersion vessel and overflow chamber connected by rubber tubing (Fig. 1). Animals suspended in cloth slings were lowered by a camera boom until the hind limb to be measured was submerged to a line painted on the leg. Water that subsequently spilled from the overflow chamber was collected in a 100-ml beaker and weighed on a microbalance to determine limb volume.

Reliability of the limb-volume measurements was determined by performing 30 repeated limb-volume measurements on each of four untraumatized frog hind limbs. Estimation of reliability using analysis-of-variance (ANOVA) procedures yielded an intraclass correlation coefficient of .995. The HVPC was supplied by four identical Intelect 500S stimulators in accordance with the manufacturer's guidelines. Output consisted of pulses delivered at 120 pps. Each pulse consisted of two fast-rising phases of 5 and 8 microseconds' duration separated by an interphase interval of 75 microseconds. The cloth slings used to suspend the frogs were lined with a 9×3.6-cm carbon-rubber electrode, which contacted the skin of the animals' abdominal wall and functioned as a cathode. Self-adhering carbon-rubber electrodes were applied to the inside walls of the beakers (one beaker for each hind limb) so that the water in the beakers served as an anode.

Procedure

Each frog was anesthetized by immersion in an aqueous solution of MS222 (3-aminobenzoic acid ethyl ester), prepared by adding 3 g of the anesthetic powder to 1 L of water. Anesthesia was maintained throughout data collection by dripping this solution onto the cloth support slings during rest periods. Lines were painted on the frogs' legs 1 cm distal to the
fibular heads prior to initial limb-volume measurements. Each hind limb was injured by dropping a steel rod onto the plantar aspect of the foot just distal to the malleoli.

A series of four 30-minute HVPC treatments was begun within 10 minutes after injury; a 30-minute rest period followed each treatment. At the commencement of each treatment, the frogs’ hind limbs were immersed in separate beakers and voltage was slowly increased until minimal movement was observed in both limbs (ie, visible motor threshold was determined). One limb, which was randomly selected, then received anodal 120-pps HVPC at 90% of that motor threshold (between 30 and 40 V, yielding pulse charges of 0.75 to 1.0 μC, respectively); the other limb, having been briefly brought to motor threshold, received no further ES and thus served as a control. The brief contraction was induced in the control limb to match the contraction induced in the treated limb, thereby eliminating the possibility of differential “muscle-pump” effects. Animals were suspended in the slings with their hind limbs in a dependent position throughout data collection.

Limb-volume measurements were taken before and after trauma and after each treatment and rest period by immersing each hind limb to its premarked line. All data were collected by a rater who was blinded to the treatment assignment. Skin was incised at each animal’s ankles and feet just before sacrifice to confirm that limb-volume changes were attributable to edema formation and not to frank bleeding.

**Data Analysis**

Data were expressed as changes from pretrauma hind-limb volumes per kilogram of body weight. An ANOVA for repeated measures was used to test the null hypothesis that treatment would not influence posttraumatic limb volumes. A 0.05 level of significance was selected.

**Results**

Anodal HVPC did not affect posttraumatic hind-limb volumes in this study. Mean volumes of the treated and untreated limbs increased in parallel fashion (ie, virtually symmetrical bilateral edema occurred); therefore, no treatment effect was evident ($P=.9011$) (Table, Fig. 2). Inspection of trauma sites revealed subcutaneous bleeding in 5 of the 28 limbs, but the blood was always diffuse and unclotted, suggesting that frank bleeding was not the primary cause of limb-volume change; that is, large blood clots or large amounts of bleeding were not responsible for the recorded swelling.

**Discussion**

Anodal HVPC did not curb posttraumatic edema formation in this study. Indeed, the probability value we obtained ($P=.9011$) was so high relative to our selected level of significance ($P<.05$) that we terminated data collection after treating only 14 animals instead of the planned 20, because we judged it highly improbable that a significant treatment effect would be discerned with data from 6 additional frogs. We believe this negative result is of considerable interest. Because the methods used in this study were nearly identical to those used in several previous studies of the effects of HVPC on edema formation, some potentially valuable comparisons are possible. A previous study conducted in our laboratory showed significant treatment effects in this same animal model when electrical stimuli of the same type were applied via a cathodal immersion technique. Furthermore, other closely related studies have corroborated the capacity of such cathodal HVPC to curb edema formation in frogs after simulated ankle sprains, when treatment is delayed 4.5 hours after impact injuries, and even when a single 30-minute treatment is applied after impact injuries. Thus, cathodal HVPC has repeatedly been shown to significantly curb edema formation in frogs early after mechanical injuries, whereas in this study, anodal HVPC did not curb edema formation. We conclude that, in the context of using HVPC at intensities 10% less than visible motor threshold for edema control, polarity of ES is an important variable.

The conclusion that polarity is an important treatment variable contradicts a recommended protocol in the operator’s manual provided with the type of HVPC generator we used. That manual recommends use of either positive or negative polarity for joint swelling and soft tissue injuries. Alon, the author of the protocol, cautions that the guideline is “initial” and not necessarily based on scientific reports. Alon and De Domenico, however, also state that polarity of leads may be important in only a couple of clinical protocols related to wound healing.

The reasons for the apparent importance of polarity of HVPC are unknown, but Alon and De Domenico postulated that an electrical field phe-
Figure 2. Mean changes in treated and untreated frog hind-limb volumes over time. Vertical lines depict standard errors. Means of treated and untreated limb volumes are not significantly different. The first pair of means are immediate posttrauma values; thereafter, pairs of means represent data collected after each 30-minute treatment and intervening 30-minute rest periods.

nomenon may occur when monophasic pulsed current (e.g., HVPC) is applied. In this scheme, polarized electrical fields are thought to force migration of charged proteins (that accumulate in interstitial fluid as a concomitant of edema formation) into lymphatics. Moreover, Alon and De Domenico offered this “electrical field theory” as a physiologic rationale for protocols designed for edema control via sensory-level HVPC. Because the duty cycle of HVPC is only about 0.16% at 120 pps and proportionately less at lower frequencies, however, iontophoretic (electrical-field) effects may be immeasurably small during application of HVPC at intensities less than motor threshold. Furthermore, because HVPC pulse durations are so short, any polar influences that may be exerted by a given pulse must be very short-lived. We found no evidence in this study that edema was occurring (Fig. 2).

Newton and Karselis rejected the hypothesis that acid and base formation occurs under electrodes, because they found no significant pH changes in the skin of nondisabled human subjects immediately after a 30-minute treatment of 100-V, 80- to 82-pps HVPC. Thus, there is little or no evidence that an electrical field phenomenon exists in sufficient magnitude to be operational in HVPC, and, even if it does, it may simply attract or repel charged interstitial proteins, without causing increased or net directional motion. If this is the case, then HVPC would not increase the likelihood that mobile interstitial proteins would find their way into lymphatics, as expected by Alon and De Domenico.

Unfortunately, without the electrical field theory, we currently have no explanation for the apparent importance of polarity of HVPC. Reed provided evidence that suggests HVPC reduces permeability of microvessels to plasma proteins. Nannmark et al, however, observed that stimulation via direct and alternating currents (5-50 μA) causes, after a delay of 30 to 160 minutes, increased permeability of the microvasculature, allowing the escape of macromolecules and even white blood cells. Strangely, however, Nannmark et al reported no evidence of edema in histological sections of stimulated tissue in which large numbers of white blood cells were visible in the extravascular spaces. Thus, Reed and Nannmark et al appear to have reported contradictory responses of the microvasculature to ES.

The forms of ES delivered to hamster cheek pouches by Reed and Nannmark et al (HVPC versus low voltage direct and alternating currents, respectively), duration of ES, and time of observation after commencement of ES, however, all differed between these studies. Nannmark et al noted increased permeability only after a minimum of 30 minutes after ES commenced (at 5 μA and up to 160 minutes at 50 μA), whereas Reed completed his observations within 5 minutes of commencement of ES, with simultaneous application of histamine. It is uncertain, therefore, whether Reed would have noted increased microvascular permeability had he continued observations. We treated our frogs with anodal HVPC for a total of 2 hours, but with rest periods interspersed. We did not, however, observe any increase in the volumes of treated limbs relative to control limbs, as would be expected by the electric field theory discussed earlier or if ES of this type promoted increased microvascular permeability.

We speculate that cathodal HVPC applied soon after injury may curb edema formation by retarding or reducing leakage of plasma proteins from intact microvessels adjacent to an injury site. Some edema formation would nonetheless be expected, because of disruption of microvessels at the injury site, with associated spilling of plasma proteins and reduction in
colloid osmotic pressure. These factors would explain our inability to wholly prevent edema formation by applying HVPC immediately after injury or to reduce absolute levels of extant edema when HVPC was applied 4.5 hours postinjury.5-7 Whatever cathodal HVPC may do to reduce the permeability of microvessels seems not to be done with anodal ES under otherwise similar conditions. Mechanisms of action are unknown and warrant further study.

The clinical implications of this and other studies using frogs as models must be tempered by the recognition that responses to trauma and HVPC treatment may differ between frogs and humans. The fact that frogs are poikilothermic (ie, cold-blooded) should not have affected the responses that we measured, because the animals were maintained at room temperature throughout data collection and the research design permitted statistical comparisons to contralateral (control) limbs, which were handled in precisely the same manner as treated limbs, except for application of electrical stimuli. Thus, we believe that it is reasonable to formulate clinical hypotheses based on our findings.

Conclusion

Even an aggressive series of anodal HVPC treatments at 120 pps and at intensities of 10% less than visible motor threshold did not curb edema formation in frog hind limbs in this study. This finding contrasts markedly with the clear treatment effects found with cathodal HVPC and suggests the importance of polarity in related clinical applications.

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References

1 Voight ML. Reduction of posttraumatic ankle edema with high voltage pulsed galvanic stimulation. Athletic Training Winter 1984, pp 278-279, 311.
5 Bettany JA, Fish DR, Mendel FC. Influence of high voltage pulsed direct current on edema formation following impact injury. Phys Ther. 1990;70:219-224.
15 Fish DR, Mendel FC, Bettany JA. Research design considerations in studying edema. Phys Ther. 1990;70:584-586. Author response to letter to the editor.
20 Reed BV. Effect of high voltage pulsed electrical stimulation on microvascular permeability to plasma proteins: a possible mechanism in minimizing edema. Phys Ther. 1988;68:491-495.

Commentary

I congratulate Fish et al for their work, which contributes significantly to our knowledge base on transcutaneous electrical stimulation, particularly the effect on edema formation. The systematic work of this group, including their previous publications, enable us to begin to identify the requirements and limitations of using electrical stimulation in edema management, an area of great clinical interest and ramifications.

My commentary primarily focuses on the evaluation of this study's findings in relation to several aspects of the theoretical rationale that Dr De Domenico and I proposed a number of years ago.3 The major issues include the meaning of the electrical field...