Biomechanical response to acupuncture needling in humans

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Received 23 March 2001; accepted in final form 25 July 2001

Langevin, Helene M., David L. Churchill, James R. Fox, Gary J. Badger, Brian S. Garra, and Martin H. Krag. Biomechanical response to acupuncture needling in humans. J Appl Physiol 91: 2471–2478, 2001.—During acupuncture treatments, acupuncture needles are manipulated to elicit the characteristic “de qi” reaction widely viewed as essential to acupuncture’s therapeutic effect. De qi has a biomechanical component, “needle grasp,” which we have quantified by measuring the force necessary to pull an acupuncture needle out of the skin (pullout force) in 60 human subjects. We hypothesized that pullout force is greater with both bidirectional needle rotation (BI) and unidirectional rotation (UNI) than no rotation (NO). Acupuncture needles were inserted, manipulated, and pulled out by using a computer-controlled acupuncture needling instrument at eight acupuncture points and eight control points. We found 167 and 52% increases in mean pullout force with UNI and BI, respectively, compared with NO (repeated-measures ANOVA, P < 0.001). Pullout force was on average 18% greater at acupuncture points than at control points (P < 0.001). Needle grasp is therefore a measurable biomechanical phenomenon associated with acupuncture needle manipulation.

Although acupuncture is increasingly used for the treatment of pain and other conditions (27, 37), the rational basis underlying its use remains unclear (2). Western medical experts have been inherently skeptical of acupuncture’s therapeutic value. One reason is that it seems very unlikely that the simple act of inserting fine needles into tissue could elicit any effect at all, let alone wide-ranging and long-lasting therapeutic effects. Hypodermic needles are routinely used in Western medicine, and their insertion into the body is not considered therapeutic. Acupuncture needles are of a finer gauge than even the finest needles used for intradermal injections, and acupuncture rarely results in a single drop of blood being discharged.

What is not widely appreciated by nonacupuncturists, however, is that acupuncture typically involves manual needle manipulation after needle insertion (3, 6, 16, 33, 40). Manual needle manipulation consists of rapidly rotating (back-and-forth or one direction) and/or pistoning (up-and-down motion) of the needle. Needle manipulation can be brief (a few seconds), prolonged (several minutes), or intermittent depending on the clinical situation (33). Even when electrical stimulation is used (a relatively recent development in the history of acupuncture), a certain amount of manual needle manipulation is usually performed immediately after needle insertion (6, 40).

Traditionally, manipulation is performed to elicit the characteristic reaction to acupuncture needling known as “de qi.” De qi has a sensory component perceived by the patient as an ache or heaviness in the area surrounding the needle and a simultaneously occurring biomechanical component that can be perceived by the acupuncturist (3, 6, 10, 16, 33). We refer to this component as “needle grasp.” During needle grasp, the acupuncturist feels as if the tissue is grasping the needle such that there is increased resistance to further motion of the manipulated needle (6, 10, 38, 40). This “tug” on the needle is classically described as “like a fish biting on a fishing line” (48). Needle grasp can range from subtle to very strong, with pulling back on the needle resulting in visible tenting of the skin (16, 21). During acupuncture treatments, needle manipulation is used to elicit and enhance de qi, and de qi is used as feedback to confirm that the proper amount of needle stimulation has been used.

De qi is widely viewed as essential to acupuncture’s therapeutic effectiveness (6, 10, 16, 23, 33, 40). Documentation of de qi has been used as a criterion for evaluating the adequacy of both manual and electrical acupuncture treatments in clinical trials (13, 46). Needle manipulation, de qi, and needle grasp, therefore, are potentially important components of acupuncture’s therapeutic effect, yet the mechanisms underlying de qi and needle grasp are unknown.

As a first step toward understanding the physiological and therapeutic significance of de qi, we have quantified needle grasp by measuring the force necessary to pull an inserted acupuncture needle out of the tissues (pullout force). We hypothesized that pullout force is greater with two different types of needle
manipulation commonly used in acupuncture practice [bidirectional (BI) and unidirectional (UNI) needle rotation] than with needle insertion with no manipulation (NO). If proven true, this will demonstrate that needle manipulation has measurable biomechanical effects. These measurable effects, together with the historical importance of this technique, will suggest that needle manipulation may indeed play an important role in acupuncture therapy. Since de qi is traditionally believed to be greater at “acupuncture points,” we also hypothesized that pullout force is greater at classically defined acupuncture points than at nonacupuncture control points.

To test these hypotheses, we carried out an experiment in which normal human subjects received different types of acupuncture needle manipulation at eight acupuncture points and eight corresponding control points. A computer-controlled acupuncture needling instrument was fabricated and used to perform all needling procedures (needle insertion, manipulation, and pullout) as well as measurement of pullout force. Needle-insertion depth was standardized and based on tissue measurements made by ultrasound.

METHODS

Study Site and Participants

The study was conducted at the University of Vermont General Clinical Research Center between June 2000 and December 2000. Healthy volunteers aged 18–55 yr were invited to participate. Exclusion criteria were a history of diabetes, neuromuscular disease, bleeding disorder, collagen vascular disease, acute or chronic corticosteroid therapy, and extensive scarring or dermatological abnormalities in the areas tested. Volunteers taking anti-inflammatory or anti-histamine medications were asked to discontinue their use 3 days before testing. Female volunteers were excluded if they were pregnant. Testing was not scheduled during menstruation to avoid possible discomfort due to cessation of anti-inflammatory medication.

Study Protocol

Study protocol was approved by the University of Vermont Institutional Review Board. Protocol summaries were mailed to volunteers for review, and written, informed consent was obtained on the day of the study. Each enrolled volunteer participated in one testing session lasting 2–3 h, during which a total of 16 points on the body received acupuncture needling. After consenting, each subject was randomized into one of three experimental groups. These groups differed only in type of needle manipulation used (BI, UNI, or NO).

Eight traditional acupuncture point locations were investigated (Fig. 1). For each location, a pair of corresponding acupuncture points on the right and left sides of the body were identified and marked with a skin marker (16 acupuncture points total). Acupuncture points were identified by an experienced acupuncturist (H. M. Langevin) according to traditional methods. Approximate position was determined in relation to anatomic landmarks (e.g., bones, tendons) and proportional measurements (e.g., fraction of the distance between wrist and elbow creases) (6). Within the area delineated by these landmarks, the precise position of each acupuncture point was determined by palpation, feeling for a slight depression or yielding of tissues. For each location, right and left sides of the body were then randomly selected for acupuncture point and control point. On the side selected for control point, a disk-shaped template was centered on the acupuncture point. The disk was 2 cm in radius for points located on the forearm and lower leg and 3 cm in radius for points located on the upper arm and thigh. The control point was marked on the perimeter of the disk at a 45° angle from the acupuncture point’s meridian and as far as possible from the nearest bone and joint. On the side selected for acupuncture point, a similar “dummy” procedure was performed and then disregarded. Each acupuncture point was therefore paired with a corresponding control point on the opposite side of the body. The term “acupuncture/control location” is hereafter used to refer to a corresponding pair of acupuncture and control points.

Throughout testing, subjects were neither told nor able to see or hear any indication of which side was used for each point (acupuncture and control) and which needle manipulation type (NO, BI, or UNI) was being performed.

Determination of Needle Insertion Depth

For each acupuncture/control location, target needle insertion depth was determined based on ultrasound measurement of subcutaneous tissue thickness. With ultrasound imaging, the perimuscular fascia is visible as an echogenic line separating two tissues of different echogenicity and compressibility (subcutaneous tissue vs. muscle). Ultrasound imaging was performed with an Acuson 128 ultrasound machine (Acuson, Mountain View, CA) equipped with a 7-MHz linear array transducer. The transducer was always held perpendicular to the skin. The same needle depth (D) was used for both acupuncture point and corresponding control point and was calculated as: $D = S + 1.5$ cm, where $S$ was the subcutaneous tissue thickness measured by ultrasound at
the acupuncture point. This formula for needle depth was based on compiling needle depth guidelines for the listed acupuncture points from seven different acupuncture textbooks (1, 3, 6, 12, 33, 39, 47) and averaging the suggested upper and lower limits of the listed ranges for each point. In a pilot study of subcutaneous tissue measurements in 16 subjects (8 men and 8 women), needle depth determination using the above formula fell within the recommended ranges at each acupuncture point in all subjects.

Acupuncture Needling

Needling system. All needling procedures (insertion, manipulation, pullout, and pullout-force measurement) were performed by a computer-controlled acupuncture needling system. This ensured consistent experimental conditions and eliminated many potential sources of investigator bias. This system consists of a hand-held needling instrument (Fig. 2), a personal computer fitted with a servomotor controller, and custom-written control and data acquisition software. The needling instrument contains two miniature servomotors, both of which are controlled by the computer. The first motor is coupled to a ball leadscrew and generates linear needle motion (needle insertion and pullout). The second motor generates needle rotation (manipulation). A 500-g capacity strain-gauge loadcell measures all axial forces exerted by the tissue on the needle.

To perform a pullout test, the system operates in the following manner. The investigator holds the instrument against a subject’s skin in the appropriate location and oriented perpendicular to the skin. Just enough force is applied to maintain light contact with the skin. The loadcell is physically isolated from the skin-contacting foot, and therefore loadcell readings are not affected by pressure against the skin by the foot of the instrument. Applying too much pressure, however, can compress the underlying tissue and could potentially influence how the tissue responds to needling. In evaluation tests, we found that the investigator could easily maintain skin contact without causing visible skin compression throughout a pullout test, and within this range no significant tissue compression artifact was found.

After the instrument has been properly positioned and oriented but just before the needling procedure has been initiated, the loadcell reading is tared. This is necessary because the weight of the needle rotation motor and needle grip (which are mounted to the loadcell’s live side) is significant compared with typical pullout force. Taring the system with the instrument in its final orientation compensates for this gravity-induced loadcell signal such that only those forces exerted by the tissue on the needle are recorded.

Once the instrument has been positioned and the loadcell tared, needling procedure is initiated. Under the computer’s control, the needle is robotically advanced into the tissue through a hole in the instrument’s skin-contacting foot (Fig. 2, inset), rotated to perform manipulation (if called for), and, after a 10-s delay, pulled out of the tissue.

Fig. 2. A: design schematic of acupuncture needling instrument. From left to right: cutaway view with needle extended, cutaway view with needle retracted, side view. B: needling instrument in use. Inset shows needle in extended position.
Needling parameters. Because the needling instrument is computer controlled, all motion parameters (e.g., insertion depth, insertion speed, amount of rotation, direction of rotation, rotation speed, dwell time, pullout speed) can be independently set. In this study, the number of needle rotations for needle manipulation was 16 clockwise for UNI and 16 alternating clockwise and counterclockwise cycles of four rotations each for BI. All other needling parameters with the exception of needle insertion depth (see above) were held constant across all points and all subjects (Fig. 3): needle insertion speed was 10 mm/s; rotation speed was 8 revolutions/s; needle dwell time was 2 s before manipulation and 10 s after manipulation; pullout speed was 5 mm/s. These parameters were determined by observing and simulating needle manipulations performed by an acupuncturist trained in a variety of different acupuncture needling techniques (H. M. Langevin). Needle-manipulation techniques vary widely in clinical practice, ranging from almost no manipulation to rapid and forceful needle movements. In this study, needle manipulations corresponding to “moderate” practice were chosen for BI and UNI.

Needling protocol. After ultrasound imaging was performed, the appropriate insertion depth for each acupuncture/control location was entered into the computer. Because needle insertion depth at each control point was set according to ultrasound measurement at the corresponding acupuncture point and to minimize repositioning of the subject between marking and needling, acupuncture points were needled before control points within each acupuncture/control location. For each point, a new sterile disposable needle (Seirin, Shimizu, Japan) 30, 40, or 50 mm in length and 0.25 mm in diameter was mounted in the needling instrument. Skin at each point was disinfected with alcohol. The needling instrument was then held by hand against the skin using just enough pressure to maintain light contact with the skin to avoid any visible compression of skin by the instrument (verified by an observer). Activation of a push-button switch initiated the needling procedure as described above. Between test points within the same subject, the instrument was disinfected by submerging in isopropyl alcohol for 30 s. Between subjects, all parts of the instrument that came in contact with the subject or the needle were steam sterilized.

Outcome Measure

The needle-grasp component of de qi is an increase in the gripping of the acupuncture needle by local tissues. Pullout-force outcome measure quantifies the force required to overcome the attractive forces between needle and tissue. During the entire needling procedure, the data acquisition system continuously recorded the needle force detected by the load-cell. The peak force occurring during the pullout phase was automatically identified and saved as the pullout-force outcome measure (Fig. 3).

Statistical Methods

Subjects randomized to the three needle-manipulation types were compared with respect to age, gender, and body mass index (BMI) by using ANOVA, x²-tests, and Kruskal-Wallis tests, respectively. Repeated-measures ANOVA was used to assess differences in mean pullout force and needle-insertion depth between acupuncture and control points and across the three needle-manipulation types. Experimental design was treated similar to a split-plot with subjects randomized to one of the three needle-manipulation types (whole plot) and acupuncture and control points (subplot) randomized to right or left side for each acupuncture/control location within subjects. Pairwise comparisons among means, when appropriate, were performed by using Fishers least significant difference (LSD) test. Data corresponding to pullout force were log transformed before analysis to satisfy the normality and homogeneity of variance assumptions associated with ANOVA (5). All means presented for pullout force are geometric means, which correspond to the antilog of the arithmetic means of the log-transformed data. Approximate standard errors associated with geometric means were computed based on the method described by Kendall and Stuart (18). Statistical analyses were performed using SAS statistical software.
RESULTS

Study Participants

Sixty-one volunteers were enrolled in the study. One female participant withdrew during testing because of discomfort associated with the testing procedure. The remaining 60, consisting of 38 women and 22 men, completed the testing protocol. Means and SD for age and BMI of participants that completed the study were 37.1 ± 10.2 yr and 26.5 ± 5.3 kg/m², respectively. There were no significant differences with respect to these subject characteristics between the groups of subjects randomized to the three needle-manipulation types.

Pullout-Force Measurements

Pullout force, the primary outcome measure, is graphically displayed in Fig. 4. Significant differences in pullout force were observed across the three needle-manipulation types [F(2,57) = 75.5, P < 0.001; Fig. 4A]. Mean pullout force (±SE) for UNI (97.5 ± 5.5 g) was significantly greater than that for BI (55.7 ± 1.7 g), and the latter was significantly greater than that for NO (36.5 ± 0.8 g) (Fishers LSD, P < 0.05).

Mean pullout force was also significantly greater at acupuncture points than at corresponding control points [F(1,57) = 18.0, P < 0.001; Fig. 4B]. Mean pullout force at acupuncture points was 63.5 ± 2.3 g compared with 53.9 ± 2.0 g at control points.

There was no evidence that differences between needle-manipulation types were dependent on point type (i.e., acupuncture vs. control) [F(2,57) = 0.73, P = 0.48 for needle manipulation by point-type interaction]. Conversely, differences in pullout force between acupuncture and control points not were dependent on the type of needle manipulation.

Secondary analyses were performed comparing pullout force within needle-manipulation types and within point types (acupuncture vs. control; Fig. 4C). Within acupuncture and control points, significant differences were found among the manipulation types [F(2,57) = 62.8, P < 0.001 for acupuncture points and F(2,57) = 49.4, P < 0.001 for control points]. Pairwise comparisons indicated that each manipulation type was significantly different from the others (Fishers LSD, P < 0.05). Acupuncture and control points also differed significantly within NO [acupuncture point: 38.7 ± 1.2 g; control point: 34.7 ± 1.1 g; F(1,19) = 26.5, P < 0.001], BI [acupuncture point: 60.5 ± 2.3 g; control point: 51.8 ± 2.4 g; F(1,19) = 9.0, P = 0.007] and UNI [acupuncture point: 109.3 ± 8.4 g; control point: 87.2 ± 7.0 g; F(1,19) = 4.9, P = 0.039].

Although the testing of individual acupuncture/control locations was not the aim of our study, a greater average pullout force was observed at acupuncture points than at control points in seven out of the eight locations tested (Ht2, Li11, Li4, Lu6, Sp6, St36, and B57), with three (Ht2, Li11, and Sp6) achieving statistical significance (P < 0.05).

DISCUSSION

Our measurements of pullout force are the first quantification of needle grasp, a biomechanical aspect
of the characteristic de qi reaction widely viewed as essential to the therapeutic effect of acupuncture. We found 167 and 52% increases in pullout force with UNI and BI, respectively, compared with NO. Needle manipulation increased pullout force at both acupuncture points and control points. Although we also found an 18% difference in mean pullout force between acupuncture points and control points, the magnitude of this difference was much smaller than the difference caused by manipulation of the needle. Together, these results indicate that needle grasp is strongly influenced by needle manipulation and that this effect is not unique to acupuncture points.

The mechanism underlying needle grasp is currently unknown. A frequently stated opinion is that needle grasp is caused by a muscle contraction (15, 40). However, the only published study supporting this view is a nonquantitative evaluation of electromyographic activity during acupuncture needling, in which needle grasp was subjectively rated by the acupuncturist (34). We believe that muscle contraction is not the source of needle grasp. Needle grasp can be observed at locations where no skeletal muscle is present (such as at the wrist) and on palms and soles where there are no arrector pili smooth muscles. Tenting of skin observed during needle grasp when the needle is pulled back also suggests that layers superficial to muscle are grasping the needle (21, 16). It is therefore likely that, although contraction of muscle may occur during needle grasp, muscle contraction is not the primary mechanism responsible for this phenomenon. Tissues likely involved in needle grasp are therefore the skin and/or subcutaneous connective tissues. Possible mechanisms involving these tissues include increased turgidity, contraction, and winding of tissue around the needle during needle rotation.

Increased tissue turgidity, resulting from extravasation of protein-rich fluid, is likely to occur as a component of the triple inflammatory response to the injury created by the acupuncture needle. However, the earliest evidence of arteriolar dilation leading to protein extravasation during the triple response occurs 10–15 min after injury (9, 45), whereas needle grasp is observed within seconds of inserting and manipulating the needle. Increased tissue turgidity because of the triple inflammatory response is therefore unlikely to be the mechanism underlying needle grasp.

Contraction of connective tissue has not been studied in relation to acupuncture but is a potentially important component of the needle-grasp phenomenon. Contraction of fibroblasts, occurring over seconds to minutes and involving polymerization of soluble actin and formation of actin stress fibers, is well documented in vitro (20). Whether such rapid cytoskeletal changes in connective tissue fibroblasts can themselves result in measurable contractile forces at the tissue level is at the present unknown.

Winding of connective tissue around the needle during needle rotation is another possible mechanism contributing to needle grasp. In an electron microscopy study of debris found on acupuncture needles after insertion, manipulation, and removal, Kimura et al. (19) observed elastic and collagen fibers that were entwined around the needle. In a study using rat tissue explants, we observed a pronounced increase in the thickness of subcutaneous tissue surrounding the needle after needle rotation, with visible winding of collagen around the needle (21). A mechanism involving winding of connective tissue is consistent with our finding of greater pullout force with UNI than with BI. An estimate of needle torque could be obtained in our human subjects by measuring the electrical current delivered to the motor during the different manipulation procedures. We typically observed that, with UNI, the torque required to rotate the needle increased continuously as needle rotation proceeds. With BI, the final torque at the end of each rotation cycle progressively increased. Figure 5 shows the amount of torque developing at the needle-tissue interface during UNI (Fig. 5A) and BI (Fig. 5B). The continuously increasing torque during UNI is consistent with tissue winding around the needle (Fig. 5A). With BI, we propose that winding alternates with unwinding, but unwinding is incomplete, resulting in a gradual build up of torque in the tissue (Fig. 5B).

A mechanism involving winding of tissue is attractive because this would greatly amplify the friction force between tissue and needle (17). Pullout forces of several hundred grams represent substantial loads given the small diameter (250 μm) of the needle. Because of its self-amplifying nature, a mechanism involving winding quickly can result in strong mechanical coupling between needle and tissue. The potential importance of this effect is that, once the needle has
become mechanically coupled to the tissue, subsequent needle manipulation (either rotation or pistoning) may pull on collagen fibers, resulting in deformation of extracellular connective tissue matrix. This matrix deformation may be transduced into local cells present within connective tissue with a wide variety of downstream effects ranging from cell contraction, gene expression, secretion of paracrine or autocrine factors, and neuromodulation ofafferent sensory input (21). These effects may be prolonged and explain the perplexing claim that acupuncture treatments can have therapeutic effects lasting days to weeks and even permanently.

Our results indicate that the effect of needle manipulation on pullout force dominates over the effect of needle placement (acupuncture vs. nonacupuncture point). The difference in the magnitude of these effects may correspond to the difference between the impact of a technique (needle manipulation) vs. the impact of the substrate on which this technique is applied (the tissue into which the needle is inserted). The technique of needle manipulation appears to have pronounced effects no matter where the needle is placed. Substrate effect, on the other hand, appears to be more subtle. The difference between these two levels of effects fits with a mechanism involving tissue winding. Winding of connective tissue in response to needle rotation would be expected to occur wherever connective tissue is present but may also vary depending on local qualitative or quantitative tissue differences. This is consistent with available evidence from clinical trials. A number of early clinical trials of acupuncture compared clinical outcomes with needling of acupuncture vs. nonacupuncture points. Reviews of these studies concluded that, although many of these trials were poorly controlled, the therapeutic effect of needling nonacupuncture points appeared either equal to that of acupuncture points or intermediate between that of needling acupuncture points and that of nonneedle placebos (2, 25, 42, 44). Apparent therapeutic effects observed at control points were attributed to the “non-specific physiological effect of needling,” or to the generalized effect of noxious stimulation (“diffuse noxious inhibitory controls or DNIC”) (42, 43). Recent well-controlled clinical trials have compared the needling of acupuncture points with sham procedures using no needling or minimal needle insertion without manipulation at nonacupuncture points (24, 36). These clinical trials showed the effectiveness of acupuncture vs. a credible sham procedure but did not aim to test for differences in therapeutic response between acupuncture points and nonacupuncture points.

Attempts to identify unique anatomical and/or physiological properties of acupuncture points and meridians so far have been mostly unconvincing. Various histological structures such as neurovascular bundles (4, 30, 32) and various types of nerve endings (7, 11, 14, 26, 35) have been reported at acupuncture points. However, no histological study so far has compared acupuncture points with control points by using quantitative morphometric methods. Skin electrical conductance has been found to be lower at acupuncture points than at control points in some studies (8, 31) but not in others (28, 29). On the other hand, acupuncture points and meridians are frequently located along connective tissue planes (between muscles or between muscle and bone or tendon) (6, 39, 47). Needle grasp may therefore be slightly greater at acupuncture points because more connective tissue can wind around the needle at those points. In addition, since neurovascular bundles are located along connective tissue planes, the same amount of needle grasp may have more powerful downstream effects at acupuncture points via stimulation of these structures by the mechanical matrix deformation caused by tissue winding.

The use of a computer-controlled instrument to perform all acupuncture needling procedures is an important and novel aspect of our study, allowing controlled insertion and manipulation of needles. We chose our needling parameters to be consistent with acupuncture practice. Some differences nevertheless necessarily exist between our study protocol and clinical acupuncture. In the clinical situation, a wide variety of needling techniques are used (22), and acupuncturists modify these techniques according to the patient’s age, underlying condition, palpation of tissues, and sensations obtained during the needling itself (6, 33). Elimination of feedback-driven adjustments in needling technique was necessary in our study to test our hypotheses objectively but might, in a clinical setting, remove an important therapeutic component. Comparison of clinical outcomes obtained with acupuncture needling performed by our instrument vs. manual acupuncture would be valuable in future studies.

An important limitation of this study is that a cause and effect relationship between pullout force and therapeutic effect has not been established. This study for the first time demonstrates a link between acupuncture needle manipulation and biomechanical events in the tissue. These biomechanical events are potentially associated with long-lasting cellular and extracellular effects. Developing an understanding of these effects in future studies may eventually lead to insights into acupuncture’s therapeutic mechanisms. In the shorter term, these same effects may also provide important biological markers that can be used in clinical trials of acupuncture. Needle grasp has been described in acupuncture textbooks for over 2,000 years (27a). This study constitutes a first step toward determining the biological and clinical significance of this phenomenon.

We thank Jason A. Yandow for assistance in conducting the study and preparing the manuscript, Gale A. Weld for recruitment of volunteers, and Adam D. Riesner and Richard A. Doran for assistance with illustrations. The ongoing support of Dr. R. W. Hamill is also gratefully acknowledged.

This study was funded by the National Institutes of Health (NIH) Center for Complementary and Alternative Medicine Grant no. R01AT-00133 and conducted at the University of Vermont General Clinical Research Center at Fletcher Allen Health Care supported by NIH Center for Research Resources Grant M01RR-00109.
REFERENCES


