Journal of Orthopaedic Research
9:908–917 Raven Press, Ltd., New York

Electromagnetic Stimulation of Bone Repair: A Histomorphometric Study

Valerio Canè, *Paolo Botti, Daniele Farneti, and †Silvana Soana

*Institutes of Human Anatomy, University of Modena, Modena, *Veterinary Surgical Clinic and †Veterinary Obstetric and Gynecological Clinic, University of Parma, Parma, Italy*

Summary: The effect of pulsing electromagnetic fields (PEMFs) on bone repair was studied in principal metacarpal bones of eight adult male horses: Six horses were treated with PEMFs, and two horses were untreated. In treated horses, Helmholtz coils were applied during a 60-day period to the left metacarpal bones, bored with eight holes of equal diameter and depth, from the middiaphysis toward the distal metaphysis. Eight equal holes bored in the right metacarpal, surrounded by unactivated Helmholtz coils, were taken as controls. The two untreated horses were taken as additional control. The results of computer-assisted histomorphometric analysis indicate that (a) in diaphyseal levels, the amount of bone formed during 60 days is significantly greater ($p < 0.01$) in PEMF-treated holes than in contralateral ones and those in control horses; (b) in metaphyseal levels, PEMF-treated holes are sometimes more closed, sometimes less, as compared with contralateral holes and those in control horses; in any case the statistical analysis indicates that the symmetry in the rate of hole repair, found between the two antimeres of control horses, is not appreciable at metaphyseal levels also; (c) there was no statistically significant difference between untreated holes in PEMF-treated horses and holes in control horses, neither at diaphyseal nor at metaphyseal levels. These preliminary findings indicate that PEMFs at low frequency influence the process of bone repair on both diaphysis and metaphysis, and seem to improve the process of bone repair in skeletal regions normally having a lower osteogenetic activity, i.e., in diaphyses as against metaphyses. **Key Words:** Bone repair—Bone formation—Pulsing electromagnetic fields—Horse metacarpus.

Notwithstanding the large body of investigations carried out during the second half of this century on the relationship between bone and electricity (3–10,13,14,21,23–27,34,37,39), the effect of electric stimulation and, more recently, of pulsing electromagnetic fields (PEMFs) on bone formation and resorption remains to be defined.

In this connection it must be noted that the rate of bone repair depends on many factors: age of subject, vascularization (22,32), compact or spongy bone architecture of the skeletal region where the bone defect is located (29), mechanical stresses (1,2,31,38,40–42), diet (30,35,36), etc. Hence, in studies on the effect of a given factor (drugs, hormones, vitamins, PEMFs, etc.) on the repair of pathological or experimental bone lesions, the degree of variability of the rate at which the same lesion repairs in natural conditions must be precisely evaluated in advance.

For this reason we evaluated the effect of PEMFs on the rate of bone formation inside transcortical holes experimentally made at different levels of homotypical metacarpal bones in adult horses, whose

Received February 26, 1990; accepted April 30, 1991.
Address correspondence and reprint requests to Dr. V. Canè at Istituto di Anatomia umana normale, Policlinico, Via Del Pozzo 71, 41100 Modena (MO), Italy.

biorhythms of repair had previously been established in untreated animals. This article represents an extension of previous preliminary reports on the same subjects (11,12,15–20).

MATERIALS AND METHODS

Eight male horses of 4–5 years of age were used. With the animals under general anesthesia by gas, a series of eight transcortical holes was made, equidistant from one another and at the same level, in the lateral margin of the right and left principal metacarpal, going from the center of the diaphysis toward the distal metaphysis (Fig. 1). Altogether, 128 holes were made.

The holes were drilled orthogonally to the longitudinal axis of the bone, covered by the periosteum, with a helicoidal tip (4.5-mm diameter) working at low revolution and cooled with a jet of sterile physiological solution of Gentalyn (Schering/Plough, Comazzo-Milano, Italy). To ensure that the holes

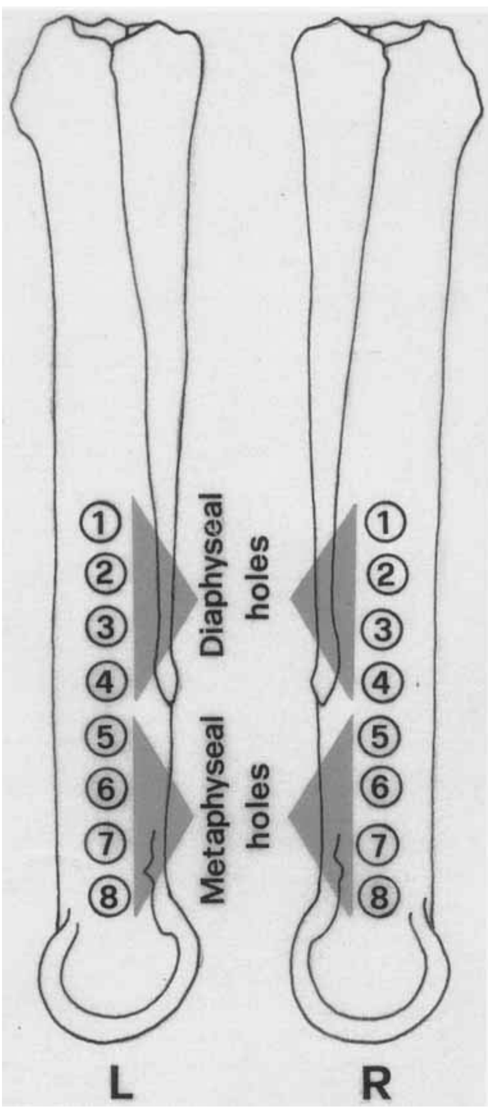

FIG. 1. Schematic drawing shows the levels where the holes were made in the left (L) and right (R) metacarpals in all horses (see text for explanation).

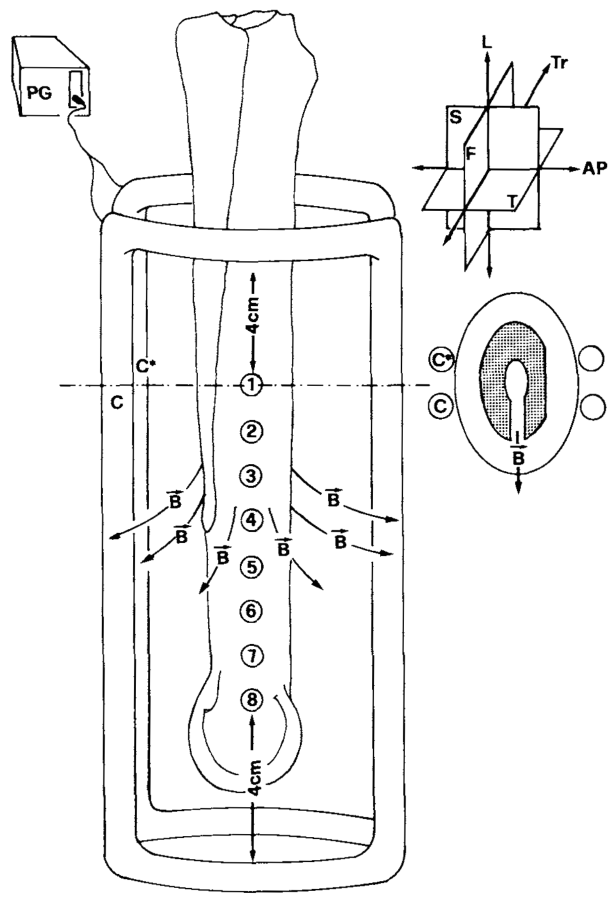

FIG. 2. Schematic drawing shows the left principal metacarpus (lateral view) surrounded by a pair of Helmholtz coils powered by a pulse generator (PG). The precise orientation of the bone is indicated on top right. L, Tr, and AP are the longitudinal, transverse, and anteroposterior axes. S, F, and T indicate the sagittal, frontal, and transverse planes. The numbers on the lateral surface of the bone indicate the levels of transcortical holes. Holes 1 and 8 are equidistant from the upper and lower ends of the coil. The two coils parallel to the sagittal plane are respectively positioned on the lateral (C) and medial (C*) aspects of the metacarpus. The central axis of each coil lies on the frontal plane passing through the holes. The arrows indicate the B field direction, which is orthogonal to the sagittal plane. The level of the cross section, drawn to the right, is indicated by the dotted line. The white elliptic band (~1.5-cm thick) between the cross sections of the bone (dotted area) and the coils (C, C*) represents the spaces occupied by the bandage and the soft tissues of the limb. The arrow in the hole indicates the B field direction.

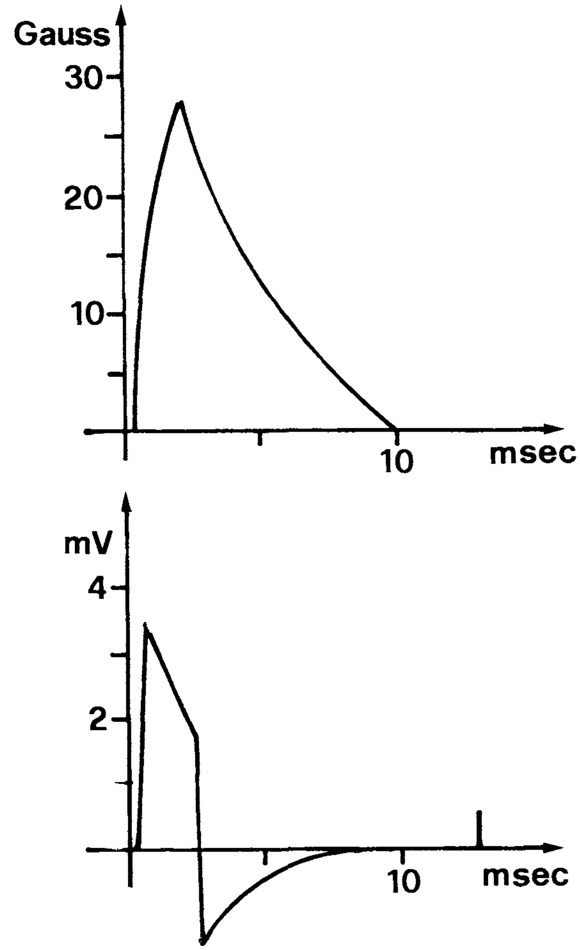

FIG. 3. **Upper diagram:** Waveform of the magnetic field used in this investigation. **Lower diagram:** Waveform of the electrical tension induced in a standard coil probe by the electromagnetic field shown above (see text for explanation).

were made at the same levels, the helicoidal tip was made to pass through a perforated steel plate, positioned in the same way in all the metacarpals.

As shown in Fig. 2, the left principal metacarpal of the six treated horses was surrounded by a pair of Helmholtz coils, each having a longitudinal diameter of 23 cm and a transverse diameter of 14 cm, and made up of 1,400 turns of copper wire (0.25-mm diameter). Both coils were continuously powered for 60 days by a pulse generator (IGEA-stimulator, IGEA, Carpi, Italy); the magnetic field signal, shown in the upper diagram in Fig. 3, was characterized by a peak amplitude of ~28 G, a rise time of 1.3 ms, and a repetition rate of 75 Hz. The lower diagram in Fig. 3 shows the waveform of the electric field, as detected by a standard coil probe made of 50 turns (0.5-cm internal diameter) of copper wire (0.2-mm diameter). No resistor was used in the standard coil probe. The peak value of the electric field induced in the probe ranged between 3.25 ± 0.25 for the position corresponding to holes 1–8. In this setup the direction of the magnetic field was perpendicular to the sagittal plane of the metacarpal bone (Fig. 2).

As control for the treated left metacarpals, we used the contralateral ones, surrounded by an equal pair of Helmholtz coils not activated by the pulse generator. Using the abovementioned standard probe, no electric field was detectable in the right limb, when the left coils were activated. In addition,

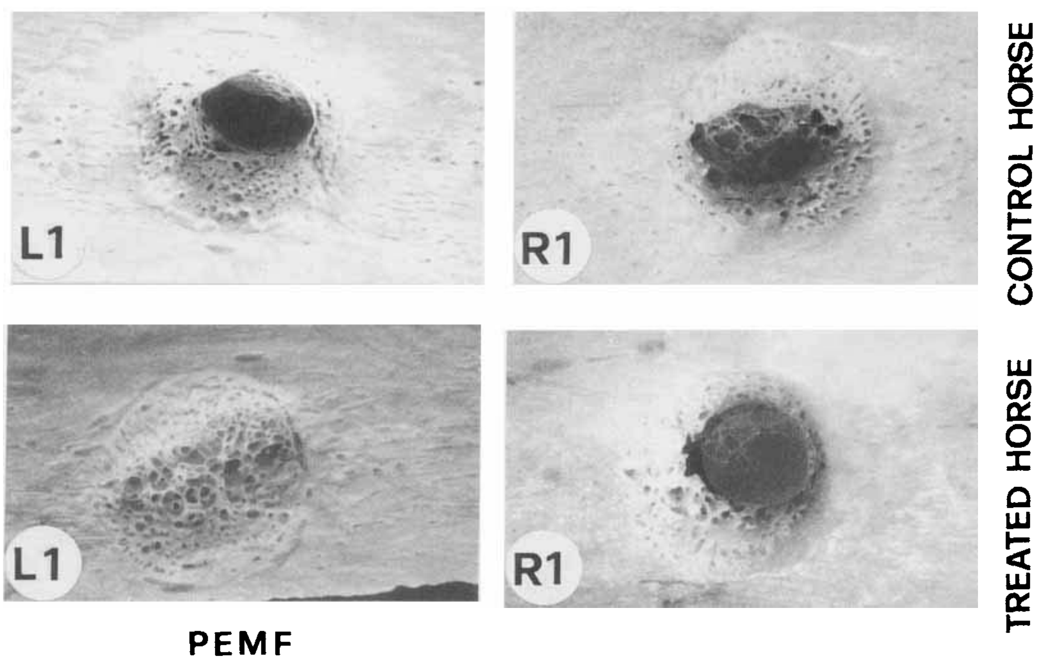

FIG. 4. Periosteal aspect of transcortical holes made at the same diaphyseal level 1 (see Fig. 1) of the left (L) and right (R) metacarpals in a control horse and in a treated one (photomicrographs under reflected ordinary light; ×6). Note that the rate of hole closure is very similar in corresponding diaphyseal levels of the control horse, whereas in the treated horse the pulsing electromagnetic field (PEMF)-treated hole (L1) is more closed as compared with both the hole in the contralateral side (R1) and those at the same level in the control horse.

the presence of the field was investigated by amplifying (×500) the sensitivity of the measurements. No field amplitude >0.02 mV was detected in the control limbs.

By way of additional control, two horses were not fitted with coils. To prevent variations in mechanical load from interfering with the repair process of the holes, throughout the period of the experiment we checked whether the surgical intervention induced changes in the normal posture of the animals. This was not the case, because both treated and untreated horses mainly assumed the standing position in the box where they were stabled. All the horses were sacrificed 60 days after application of the coils.

The bone segments containing the transcortical holes were cleaned of the soft tissues with NaOCl at 3% and embedded in methylmethacrylate. Using a diamond-blade saw (Leitz 1600 Microtome, Wetzlar, Germany), a section was removed from

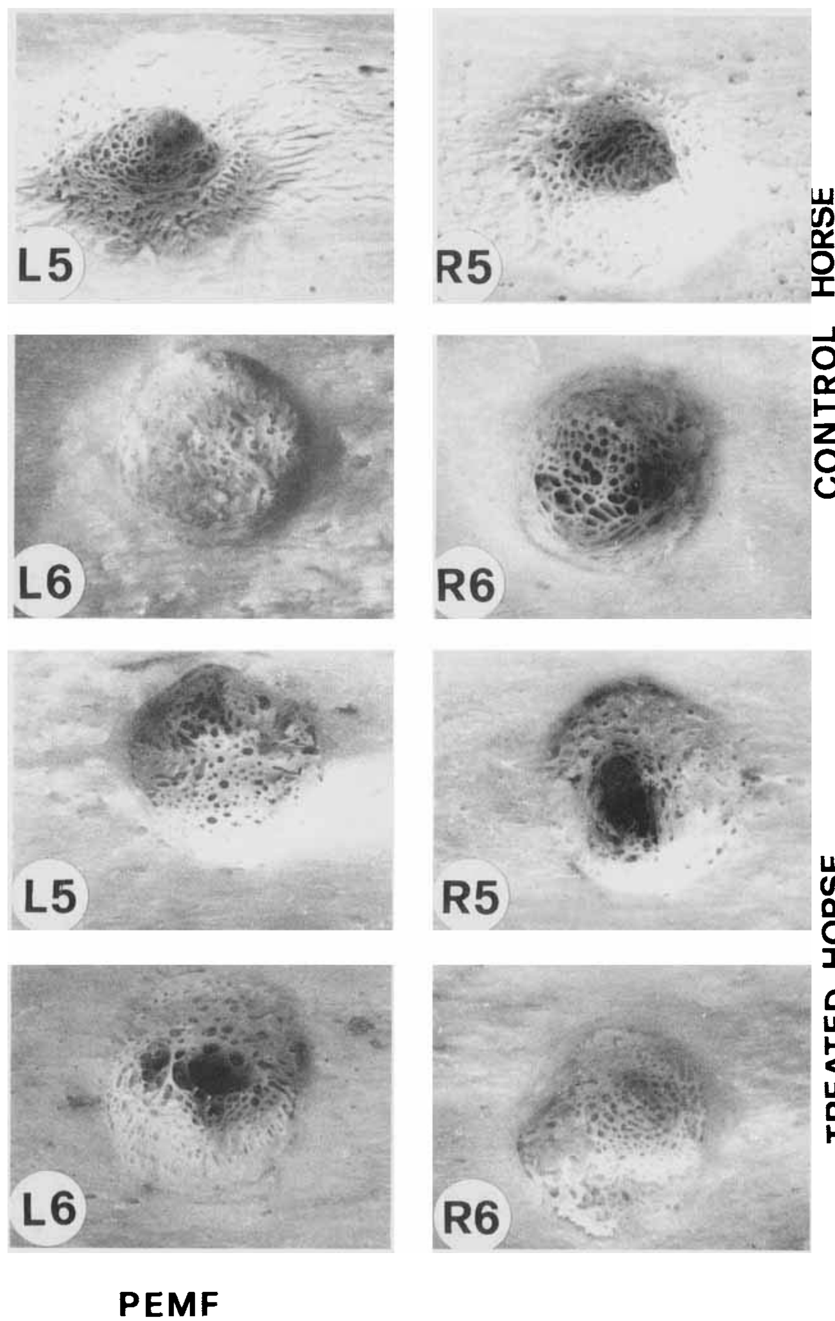

FIG. 5. Periosteal side of transcortical holes made at the metaphyseal levels 5 and 6 (see Fig. 1) of the left (L) and right (R) metacarpals in a control horse and in a pulsing electromagnetic field (PEMF)-treated one (photomicrographs under reflected ordinary light; ×5). Note in the control horse that the amount of new bone formed inside the holes is very similar in the two antimeres. In contrast, the PEMF-treated hole (L5) is more closed than the contralateral one (R5), whereas the PEMF-treated hole (L6) is less closed than the contralateral one (R6).

each segment, longitudinally with respect to the axis of the hole and passing through its center (midlongitudinal section).

All the sections ground to a uniform thickness of 70–80 μm were microradiographed (20 mA; 7.8 KV) (Italstructure Microradiograph, Como, Italy). Using a computerized image analyzer (TESAK, Florence, Italy), the following parameters were measured on the microradiographs: (a) total area of the hole; (b) area occupied by the bone it contains, i.e., the bone laid down during the 60 days of the experimental period [new bone (NB)]; and (c) area of the spaces not occupied by bone tissue [porosity (P)]. In diaphyseal holes, the bone internal to the endosteal border was excluded; moreover, to allow comparisons between the data from diaphyseal holes with those from metaphyseal ones, in the latter only their cortical portion was taken into account in the present morphometric analysis. The relative amount of bone laid down during 60 days was expressed as percentage ratio of NB/(NB + P). Statistical analyses were performed by two-tailed paired Student's *t* test.

RESULTS

The endosteal surface of the holes in the metacarpals of all the horses studied appears smooth at holes 1–4, and covered by increasingly thicker spongy bone from holes 5–8. In view of the fact that spongy bone has a higher metabolism than compact bone, our study considered as diaphyseal holes 1–4, i.e., those made only in compact bone, and as metaphyseal holes 5–8, involving both compact and spongy bone (Fig. 1).

In control horses, the 32 holes observed macroscopically from the periosteal side have a funnel-shape appearance. The rate of bone repair proceeds with a high degree of symmetry in holes situated at the same levels of the right and left antimeres (Figs. 4 and 5). Comparison between the holes situated at different levels of each antimere reveals, however, that metaphyseal holes are generally shallower than diaphyseal ones and are, to a larger extent, filled with denser trabeculae of spongy bone (Figs. 4 and 5). Light-microscope observation of the microradiographs of the midlongitudinal sections of the holes (Figs. 6 and 7) shows that new bone formation proceeds in a concentric direction, going from the endosteum toward the periosteum. The trabeculae appear predominantly oriented according to the axis of the hole and are more densely arranged and radioopaque, i.e., more mineralized and thus laid down first, in proximity to the endosteal side as opposed to the periosteal surface. Microradio-

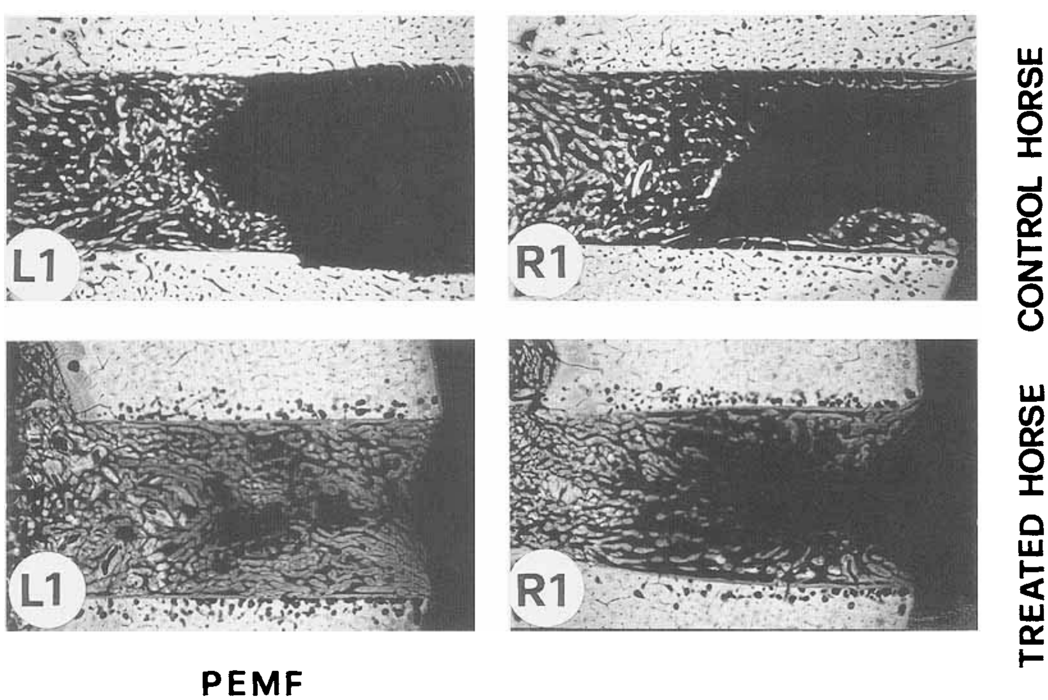

FIG. 6. Microradiographs of the midlongitudinal section of transcortical holes made at the same diaphyseal level 1 (see Fig. 1) of the left (L) and right (R) metacarpals in a control horse and in a treated one (photomicrographs under transmitted ordinary light; ×5). Although proceeding according to the same pattern, reparative osteogenesis is greater in the pulsing electromagnetic field (PEMF)-treated hole (L1) than in the contralateral one (R1) and in those at the same level in the control horse. Note also the symmetrical correspondence in the amount of bone present inside the left and right holes in the control horse.

graphic study also shows a considerable symmetry in the trend of repair of holes situated at the same level of homotypical bones. However, in metaphyseal holes, where reparative osteogenesis is more active than in the diaphyseal ones, the degree of symmetry appears sharper and subject to greater variability (Fig. 7).

In treated horses, the 96 holes observed macroscopically from the periosteal side have a funnel-shape appearance similar to that found in control horses. However, as regards the rate of repair of the holes at the same levels of homotypical bones, the symmetry found in control horses is no longer evident from the quantitative point of view. In particular, at the diaphyseal level, all the holes treated with PEMFs, as compared with the untreated contralateral holes and the holes in control horses, appear more closed by dense, spongy bone (Fig. 4). At metaphyseal levels, in contrast, the depth of the holes is sometimes greater in those treated with

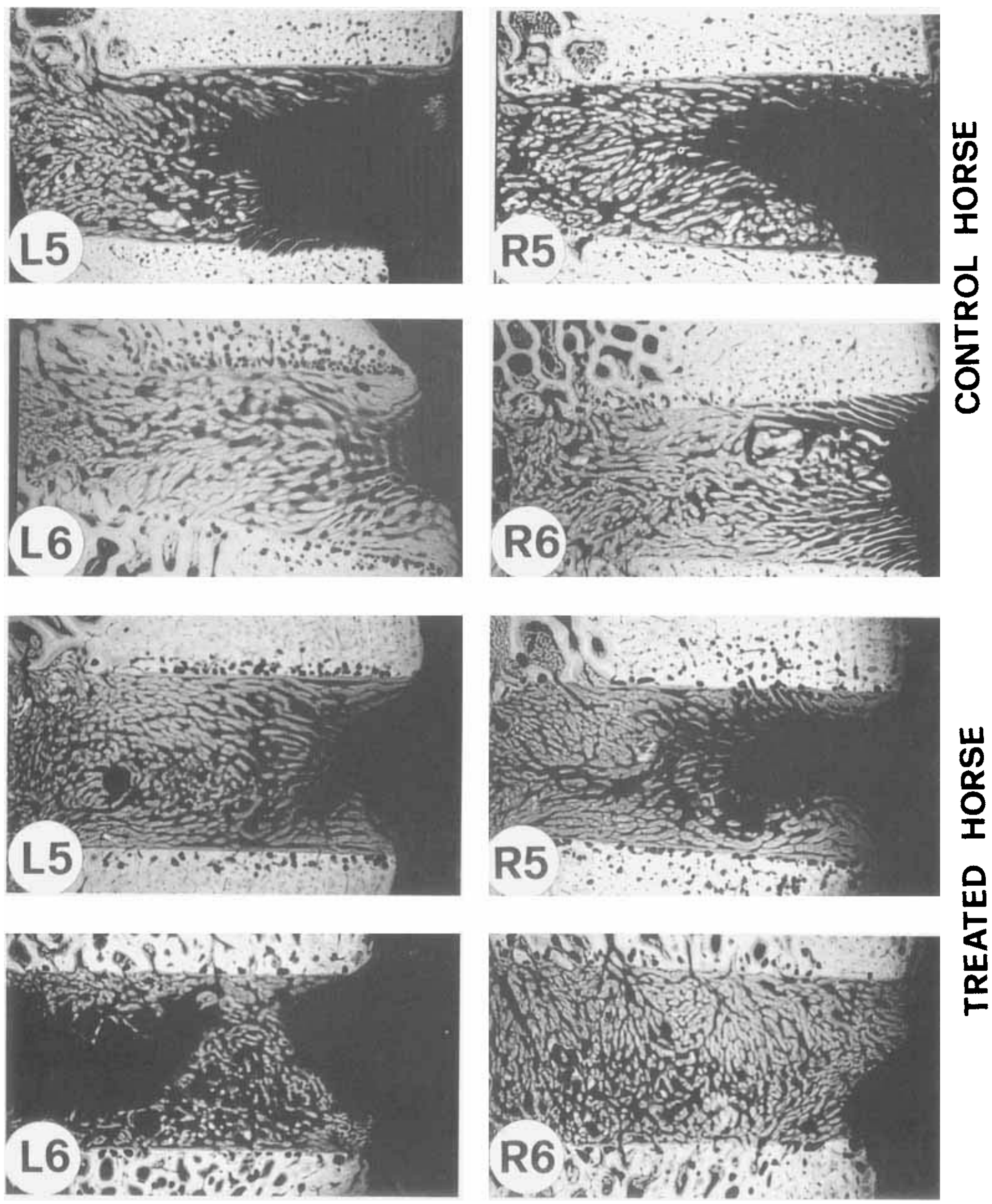

FIG. 7. Microradiographs of the midlongitudinal section of transcortical holes made at the metaphyseal levels 5 and 6 (see Fig. 1) of the left (L) and right (R) metacarpals in a control horse, and in a treated one (photomicrographs under transmitted ordinary light; ×4). In the control horse, reparative osteogenesis appears to be symmetrical in metaphyseal holes at the same levels of homotypical metacarpals. In contrast, the pulsing electromagnetic field (PEMF)-treated hole L5 is more closed than the contralateral one (R5) is, whereas the PEMF-treated hole L6 is less closed than the contralateral R6 is.

PEMFs, as compared with the contralateral holes, and sometimes is the reverse (Fig. 5). In all holes, whether treated or untreated, the newly formed spongy bone is predominantly composed of thin trabeculae in the less repaired holes and of thick trabeculae in the more closed ones. The microradiographic study also shows that in all the PEMF-treated holes the process takes place concentrically and from the endosteum toward the periosteum, as already observed in control horses. From the quantitative point of view, the microradiographs again show that the PEMF-treated holes at the diaphyseal level are at a more advanced stage of repair as compared with the controls (Fig. 6), whereas at the metaphyseal level they are sometimes more closed, sometimes less; in any case the symmetry found in control horses is not appreciable (Fig. 7).

The total amount of bone newly formed during 60 days in each hole is reported in Fig. 8.

In the two control horses, the percentage ratio NB/(NB + P) (a) increases on proceeding from the diaphysis toward the metaphysis, and (b) presents no significant differences in corresponding levels of homotypical bones. The percentage differences between the symmetrical holes in the two antimeres vary between 1.5–3% in diaphyses and 3.4–3.7% in metaphyses (Fig. 9).

In the six treated horses, the data obtained at the diaphyseal level differ substantially from those obtained at the metaphyseal level. At the diaphyseal level, the values of the percentage ratio NB/(NB + P) are significantly higher ($p < 0.01$) in PEMF-treated holes than in untreated contralateral ones, and in corresponding holes of control animals (Fig. 8), the percentage differences ranged between 40 and 120%. At the metaphyseal level, the values of %NB/(NB + P) in some PEMF-treated holes are higher, and in others are lower as compared with the contralateral ones. However, statistical analysis indicates that also at the metaphyseal level there is a significant difference ($p < 0.01$) between the values of %NB/(NB + P) recorded from holes situated at the same level of the left and right antimeres. No statistically significant difference exists between untreated holes in PEMF-treated horses and holes in control horses, either at diaphyseal or in metaphyseal levels (Table 1).

DISCUSSION

The results here reported suggest that PEMFs do not seem to alter the normal trend of the repair process of transcortical holes. In fact, in PEMF-treated holes, as in control ones, closure occurs (a) in a concentric direction and from the endosteum toward the periosteum, and (b) at a faster rate in metaphyses than in diaphyses. The latter datum probably depends on the fact that bone turnover increases on going from diaphysis to metaphysis (28,29). In this connection, it is interesting to note that the distribution of the bioelectric potentials has been shown to vary at the different levels of the long bones (33).

However, from the quantitative point of view the PEMFs do seem to exert a positive effect on the

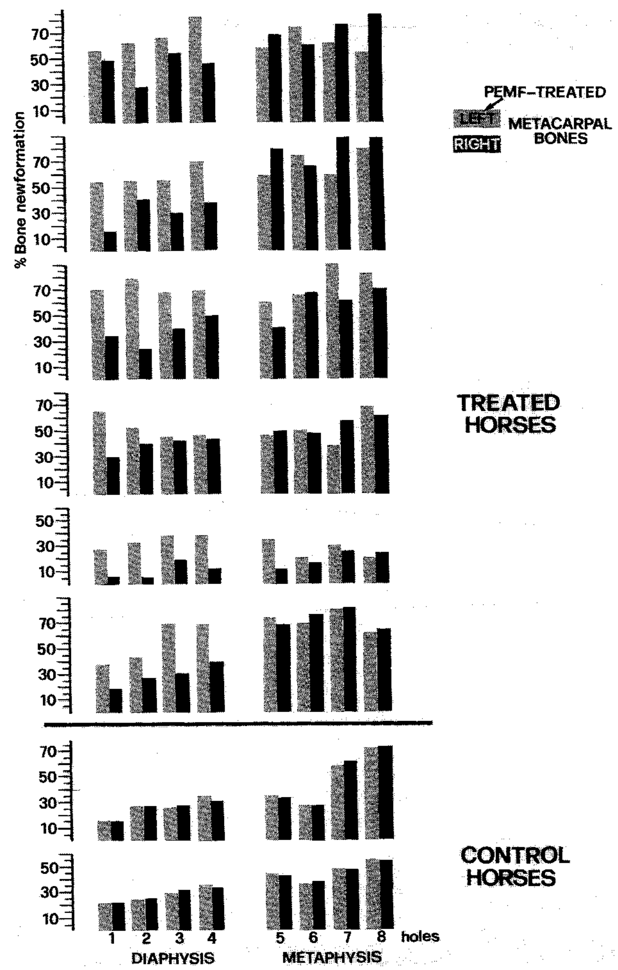

FIG. 8. Diagram of the amount of bone deposition during 60 days in transcortical holes made at the same levels in the homotypical metacarpals of eight horses (see Fig. 1). On the *x* axis, the figures indicate the level of the holes—diaphyseal region (1–4), metaphyseal region (5–8)—of the left (gray columns) and right (black columns) metacarpals in two control horses (bottom) and in six pulsing electromagnetic field (PEMF)-treated horses (top). On the *y* axis are plotted the values of %NB/(NB + P) (see text for explanation).

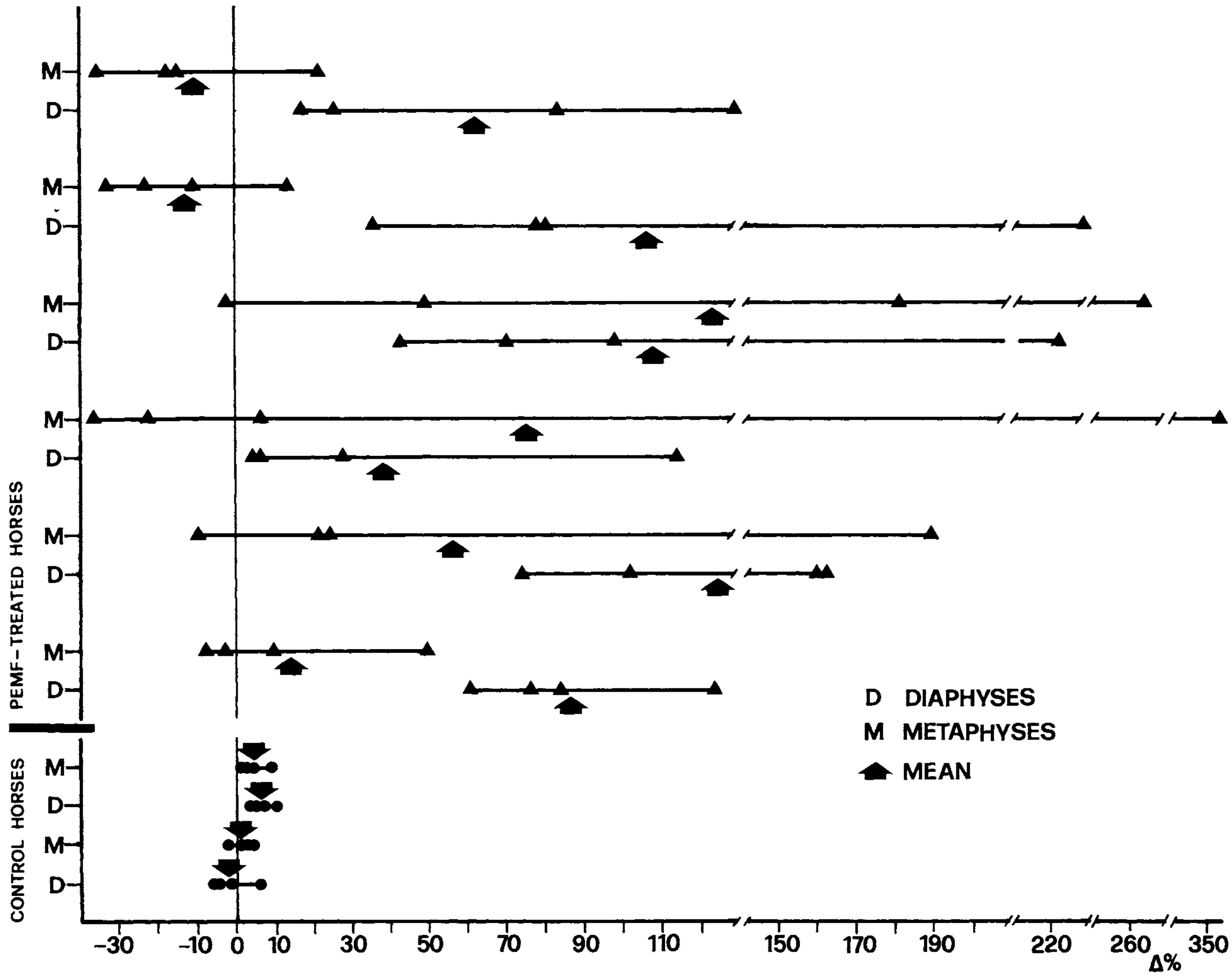

FIG. 9. Percentage differences between the left and right couple of holes (the right made equal to 100) for the parameter %NB/(NB + P). On the *x* axis, the percentage differences (the mean values are indicated by arrows); on the *y* axis, the diaphyseal (D) and metaphyseal (M) holes for control and pulsing electromagnetic field (PEMF)-treated horses. Note that the percentage differences between the left and right side are much higher in treated horses than in control ones (also see text for explanation).

repair of the holes at the diaphyseal level, whereas at the metaphyseal level their effect appears sometimes positive, sometimes negative. At the present time this PEMF-induced irregularity of the reparative osteogenesis at the metaphyseal level appears inexplicable.

On the whole, the data here reported indicate that PEMFs seem to stimulate resting or less active

TABLE 1. *Statistical analysis of the mean values of the ratio %NB/(NB + P)*

	Two untreated horses		Six PEMF-treated horses			
	8 RH	8 LH	24 RH	p	24 LH[a]	p
Diaphysis	26.9 ± 2.2	26.5 ± 2.3	32.6 ± 2.4	ns	55.9 ± 3.04	<0.0001
Metaphysis	47.1 ± 5.5	46.5 ± 5.1	54.3 ± 5.3	ns	58.9 ± 3.9	<0.1

Each mean value ± SE was calculated from the number of holes indicated before the symbols RH (right holes) and LH (left holes). NB, new bone; P, porosity; PEMF, pulsing electromagnetic fields. Note that a statistically significant difference only exists at diaphyseal levels between PEMF-treated holes of treated horses and the remaining holes, i.e., those of the contralateral side and of untreated horses. Note also that at metaphyseal levels, the degree of symmetry recorded in untreated horses between RH and LH decreases in PEMF-treated horses.

[a] PEMF-treated holes.

bone cells, but not bone cells that are already active or actively forming bone. It must be stressed, however, that bone repair is a very complicated process characterized by a sequence of events of which bone deposition represents only the final stage. Because our investigation is for the present confined to quantitative assessment of this final stage (material before 60 days has not yet been observed), we cannot rule out the possibility that the modulating effect of PEMFs may have involved the stage preceding the reparative osteogenesis rather than the latter itself. In this regard, it should be noted that the greater part of the PEMF effect on bone healing seems to occur during the early rather than the final stages of injury. It is our hope that the structural investigation currently undertaken by us, at different phases of bone repair under PEMF treatment, may contribute toward clearing up this problem.

Acknowledgment: We thank Prof. Gastone Marotti, Director of the Institute of Human Anatomy in the University of Modena, for providing positive criticism and assistance with the preparation of the manuscript; Dr. Pierluigi Fabbri, Director of C.I.G.S. (Centro Interdipartimentale Grandi Strumenti) in the University of Modena, for technical assistance in using the TESAK computerized image analyzer; and IGEA S.r.l. (Carpi-Italy) for supplying the stimulating devices. Investigation was supported by a grant (40%) from the Italian Ministry of Education.

REFERENCES

1. Amprino R: La struttura delle ossa dell'uomo sottratte alle sollecitazioni meccaniche. *W Roux'Arch Entwickl-Mech Org* 138:305–322, 1938
2. Amprino R, Marotti G: A topographic-quantitative study of bone formation and reconstruction. In: Blakwood HJJ, ed. *Bone and Tooth.* Oxford: Pergamon Press, 1964:21–33
3. Auer JA, Burch GE, Hall P: Review of pulsing electromagnetic field therapy and its possible application to horses. *Equine Vet J* 15:354–360, 1983
4. Bassett CAL: Pulsing electromagnetic fields: a new method to modify cell behaviour in calcified and noncalcified tissue. *Calcif Tissue Int* 34:1–8, 1982
5. Bassett CAL, Becker RO: Generation of electric potential by bone in response to mechanical stress. *Science* 137:1063–1064, 1962
6. Bassett CAL, Pawluck RJ, Pilla AA: Acceleration of fracture repair by electromagnetic fields. A surgically noninvasive method. *Ann NY Acad Sci* 238:242–262, 1974
7. Bassett CAL, Pawluck RJ, Pilla AA: Augmentation of bone repair by inductively coupled electromagnetic fields. *Science* 184:575–577, 1974
8. Bassett CAL, Pilla AA, Pawluck RJ: A non operative salvage of surgically-resistant pseudarthroses and non-unions by pulsing electromagnetic fields. A preliminary report. *Clin Orthop* 124:128–143, 1977
9. Becker RO: Electrical osteogenesis—pro and con. *Calcif Tissue Res* 26:93–97, 1978
10. Borsalino G, Bagnacani M, Bettati E, Fornaciari F, Rocchi R, Uluhogian S, Ceccherelli G, Cadossi R, Traina GC: Electrical stimulation of human femoral intertrochanteric osteotomies. *Clin Orthop* 237:256–263, 1988
11. Botti P, Canè V, Del Bue M, Remaggi F, Soana S: Allestimento di un modello sperimentale standard per lo studio dei processi riparativi ossei nel cavallo. *Atti 8° Congr Naz Soc It di Ippol,* Siena, Italy, 1987, pp 49–56
12. Botti P, Canè V, Remaggi F, Soana S: Studio comparativo della riparazione di fori transcorticali ed intracorticali sperimentalmente praticati in ossa omotipiche di cavallo. *Atti della Soc It Sc Vet* 41:386–389, 1987
13. Bramlage LR, Weisbrode SM, Spurlock GE: The effect of a pulsating electromagnetic field on the acute healing of equine cortical bone. *Proc Am Assoc Equine Pract* 43–48, 1984
14. Bruce GK, Howlett CR, Hucktep RL: Effect of a static magnetic field on fracture healing in a rabbit radius. *Clin Orthop* 222:300–306, 1987
15. Canè V, Botti P: Differences in the rate of closure of transcortical holes made at different levels of horse metacarpus. *Int J Orthop Surg Traumatol* 27:1–13, 1988
16. Canè V, Botti P, Del Bue M: Differenze topografiche nella velocità di riparazione di lesioni ossee sperimentalmente indotte. *Atti del 61° Congr Naz Soc It Anat,* 1986, p 19
17. Canè V, Botti P, Farneti D, Soana S: Effects of PEMFs on bone repair of transcortical holes at different levels of long bones. *Int. Symp. in Honour of Luigi Galvani on "Electrobiology Today,"* Bologna, Italy, April 14–16, 1989, p 6
18. Canè V, Botti P, Farneti D, Soana S: Histomorphometric study of low frequency pulsing electromagnetic fields (PEMFs) on bone repair of transcortical holes. *Trans Bioelect Rep Growth Soc* 9:19, 1989
19. Canè V, Botti P, Remaggi F: Studio istomorfometrico sul processo riparativo di fori intracorticali sperimentalmente indotti a livelli diversi di ossa lunghe nel cavallo. *Atti 62° Congr Soc It Anat,* 1987, p 71
20. Canè V, Botti P, Remaggi F, Marotti G: Differences in the rate of closure of standard holes made in different regions of long bones. *Calcif Tissue Int* 41(suppl 2):67, 1987
21. Collier MA, Were KE, de Freitas CB, Lamar AM: Electrostimulation of bone production in the horse. *Proc Am Assoc Equine Pract* 27:71–89, 1981
22. Enlow DH: Functions of the haversian system. *Am J Anat* 110:269–306, 1962
23. Fukada E, Yasuda I: On the piezoelectric effect of bone. *J Phys Soc Jpn* 12:1158–1162, 1957
24. Hellewell AB, Beljan JR: The effect of a constant direct current on the repair of an experimental osseous defect. *Clin Orthop* 142:219–222, 1979
25. Kold SE, Hickman J, Melsen F: Preliminary study of quantitative aspects and the effect of pulsed electromagnetic fields treatment on the incorporation of equine cancellous bone grafts. *Equine Vet J* 19:120–124, 1987
26. Law HT, Annan I, McCarthy ID, Hughes SPF, Stead AC, Camburn MA, Montgomery H: The effect of induced electric currents on bone after experimental osteotomy in sheep. *J Bone Joint Surg [Br]* 67:463–469, 1985
27. Marino AA, Cullen JM, Reichmanis M, Becker RO: Fracture healing in rats exposed to extremely low-frequency electric fields. *Clin Orthop* 145:239–244, 1979
28. Marotti G: Quantitative studies on bone reconstruction. 1 The reconstruction in homotypic shaft bones. *Acta Anat (Basel)* 52:291–333, 1963
29. Marotti G: Map of bone formation rate values recorded throughout the skeleton of the dog. In: Meunier PJ, ed. *Bone Morphometry,* University of Ottawa Press, 1976:202–207
30. Marotti G, Canè V, Sissons HA, Ling L, Kelman GJ: A light and scanning electron microscopic study of osteocyte activ-

ity in calcium deficient rats. *Calcif Tissue Int* (suppl 39):28, 1986
31. Marotti G, Marotti F: Topographic-quantitative study of bone formation and reconstruction in inert bones. In: Fleisch H, Blakwood HJJ, Owen M, eds. *Calcified Tissue 1965*, Springer-Verlag, 1966:89–93
32. Marotti G, Zambonin Zallone A: Changes in the vascular network during the formation of haversian systems. *Acta Anat* 106:84–100, 1980
33. Rubinacci A, Tessari L: A correlation analysis between bone formation rate and bioelectic potentials in rabbit tibia. *Calcif Tissue Int* 35:728–731, 1983
34. Sanders-Shamis M, Bramlage LR, Weisbrode SE, Gabel A: A preliminary investigation of the effect of selected electromagnetic field devices on healing of cannon bone osteotomies in horses. *Equine Vet J* 21:201–205, 1989
35. Sissons HA, Kelman GJ, Marotti G: Mechanisms of bone resorption in calcium-deficient rats. *Calcif Tissue Int* 36:711–721, 1984
36. Sissons HA, Kelman GJ, Marotti G: Bone resorption in calcium-deficient rats. *Bone* 6:345–347, 1985
37. Smith SD: Induction of partial limb regeneration in rana pipiens by galvanic stimulation. *Anat Rec* 158:89–97, 1967
38. Uhthoff HK, Jaworski ZFG: Bone loss in response to long-term immobilization. *J Bone Joint Surg [Br]* 60:420–429, 1978
39. van der Kuij P, Vingerling PA, Sillevis Smitt PA, de Graaf J: Reducing residual ridge reduction. *Reconstr Surg Traumatol* 19:98–105, 1985
40. Vigliani F: Accrescimento e rimodellamento strutturale della compatta in ossa sottratte alle sollecitazioni meccaniche. Nota 1. *Z Zellforsch* 42:59, 1955
41. Vigliani F: Accrescimento e rinnovamento strutturale della compatta in ossa sottratte alle sollecitazioni meccaniche. Nota 2. *Z Zellforsch* 43:17, 1955
42. Vigliani F, Marotti F, Suglia G: Il comportamento di innesti ossei sottoposti ad una sollecitazione meccanica di tipo flettente. *Boll Soc Ital Biol Sper* 43:1746–1749, 1967