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Pulsed Magnetic Fields Improve Osteoblast Activity During the Repair of an Experimental Osseous Defect

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Summary: The influence of pulsed low-frequency electromagnetic fields (PEMFs) on bone formation was investigated in studies of the healing process of transcortical holes, bored at the diaphyseal region of metacarpal bones of six adult horses, exposed for 30 days to PEMFs (28 G peak amplitude, 1.3 ms rise time, and 75 Hz repetition rate). A pair of Helmholtz coils, continuously powered by a pulse generator, was applied for 30 days to the left metacarpal bone, through which two holes, of equal diameter and depth, had been bored at the diaphyseal region. Two equal holes, bored at the same level in the right metacarpal and surrounded by an inactive pair of Helmholtz coils, were used as controls. All horses were given an intravenous injection of 25-30 mg/kg of tetracycline chloride on the 15th and again on the 25th day after the operation and were killed 5 days later. The histomorphometric analysis indicated that both the amount of bone formed during 30 days and the mineral apposition rate during 10 days (deduced from the interval between the two tetracycline labels) were significantly greater ($p < 0.01$ and $p < 0.0001$, respectively) in the PEMF-treated holes than in the controls. As did a previous investigation, these preliminary findings indicate that PEMFs at low frequency not only stimulate bone repair but also seem to improve the osteogenic phase of the healing process, at least in our experimental conditions.

Previous investigations in our laboratory on the rate of repair of transcortical holes in the shaft bones of adult horses showed that pulsed low-frequency electromagnetic fields (PEMFs) constantly exert a positive effect in the diaphyseal region, whereas in the metaphyseal region they improve or reduce the healing process as compared with controls (11,12). Our data were confined to quantitative assessment of the later stage of hole repair—bone deposition—and thus give no indication of whether the modulating effect of PEMFs may have involved the early events of the bone reparative process characterized by soft tissue; that is, hematoma, cellular granulation tissue, and soft callus.

In an attempt to solve this problem, we carried out a new series of experiments using the tetracycline labeling technique to detect whether PEMFs are involved in modulating the rhythm of bone-forming cells during the osteogenic stage of healing. This paper represents an extension of previous preliminary reports on the effect of PEMFs on mineral apposition rate (MAR) during the repair of transcortical holes at the diaphyseal level only.

MATERIAL AND METHODS

Six male horses, 8-10 years of age, were used. While they were under general anesthesia by gas (fluothane 1-2%, O_2 30%, and N_2O 68-69%), two

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transcortical holes were made, 3 cm apart and at the same level, in the lateral margin of the right and left principal metacarpals in the mid-diaphyseal region. The holes were drilled orthogonally to the longitudinal axis of the bone, with a helicoidal tip (4.5 mm diameter) working at low speed and cooled with a jet of sterile physiological solution of Gentalyn (gentamicin C complex sulfate; Schering/Plough, Comazzo-Milano, Italy).

In accordance with the methodology used in previous investigations (12), the metacarpals of both sides were surrounded by a pair of Helmholtz coils (IGEA, Carpi, Italy), which were oriented medial-lateral to the sagittal plane of the bone. The coils

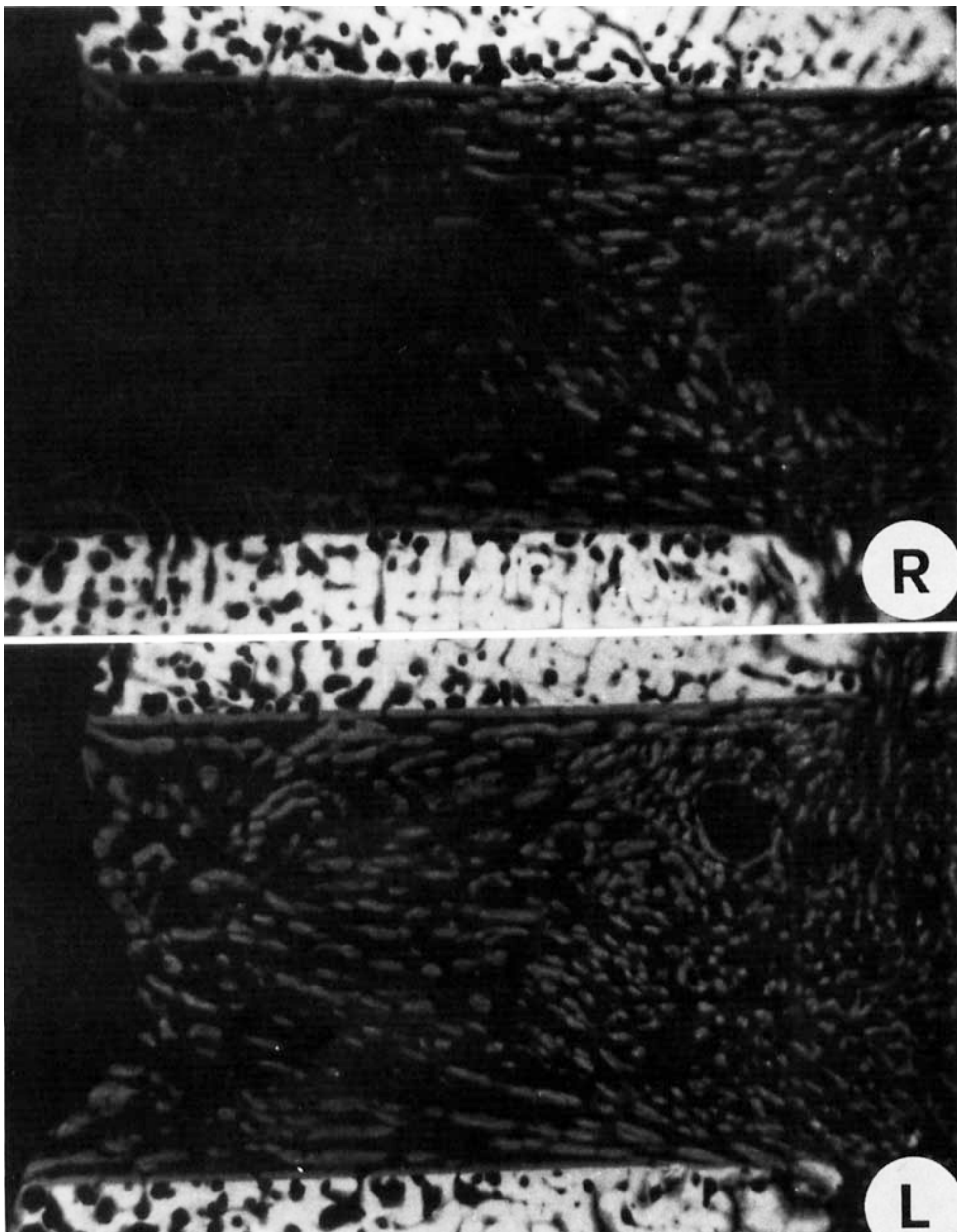

FIG. 1. Microradiographs of the midlongitudinal section of transcortical holes made at the same diaphyseal level in the right (R) and left (L) metacarpals (photomicrographs under transmitted ordinary light; ×15). The amount of newly formed bone is greater in the hole on the side treated with pulsed low-frequency electromagnetic fields (L).

surrounding the bone in the left side were powered for 30 days by a pulse generator (IGEA-stimulator; IGEA). As in a previous experiment (12), the magnetic field signal employed was characterized by a peak amplitude of about 28 G, a rise time of 1.3 ms, and a repetition rate of 75 Hz. The peak value of the electric field induced in a standard coil probe was 3.25 ± 0.25 mV in the left limb, whereas the induced electric field in the contralateral right limb, measured by means of a probe with a sensitivity level of 0.01 mV (12), was practically undetectable.

As a kinetic marker of bone mineralization, tetracycline chloride (Reverin; Hoechst AG, Frankfurt, Germany) was injected into the jugular vein at a dose of 25-30 mg/kg. All horses were given an injection on the 15th and again on the 25th day after the operation and were killed 5 days later.

The undecalcified bone segments containing the holes were cleaned of the soft tissues with NaOCl at 3% and were embedded in methylmethacrylate (BDH Italia, Milano, Italy). Sections were cut with a microtome (1600 microtome; Leitz, Wetzlar, Germany) from each segment, longitudinally with respect to the axis of the hole and passing through its center (mid-longitudinal section). Transverse hemisections with respect to the axis of the hole also were cut in proximity to the endosteal side of each hole.

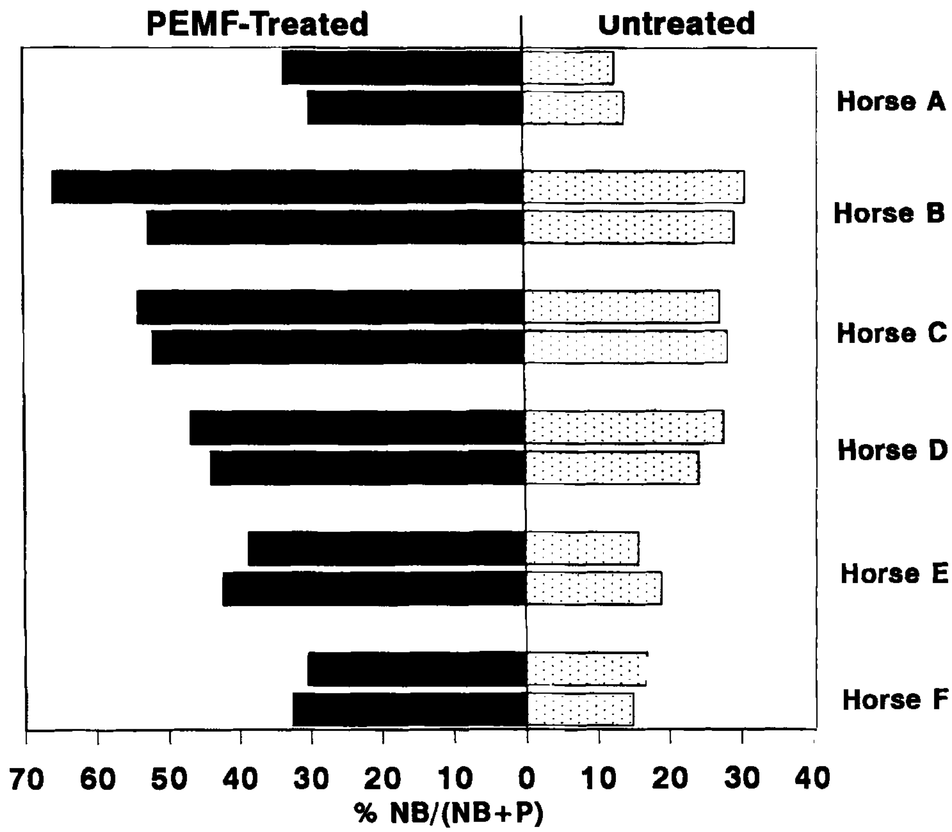

FIG. 2. Diagram of the amount of new bone formation during 30 days in two transcortical holes drilled at the same diaphyseal levels in the homotypical metacarpals of six adult horses. Vertical axis: the two holes in the metacarpals treated with pulsed low-frequency electromagnetic field (PEMF) (black bars) and the untreated metacarpals (right) (dotted bars) of each animal. Horizontal axis: the values of %NB/(NB + P), where NB is the new bone formed and P is the porosity.

All sections, each 30-40 μm thick, were microradiographed (20 mA; 7.8 kV) (Microradiograph; Italstructure, Riva del Garda-Trento, Italy) on electron microscope polyester based film (ILFORD EM; Ilford, Mobberley, Cheshire, England).

The following parameters were measured, with the use of a computer image analyzer (TESAK, Florence, Italy), on the microradiographs of the mid-longitudinal sections: (a) the total area of the hole; (b) the area occupied by the new bone (NB) it contains (that is, the bone laid down during 30 days); and

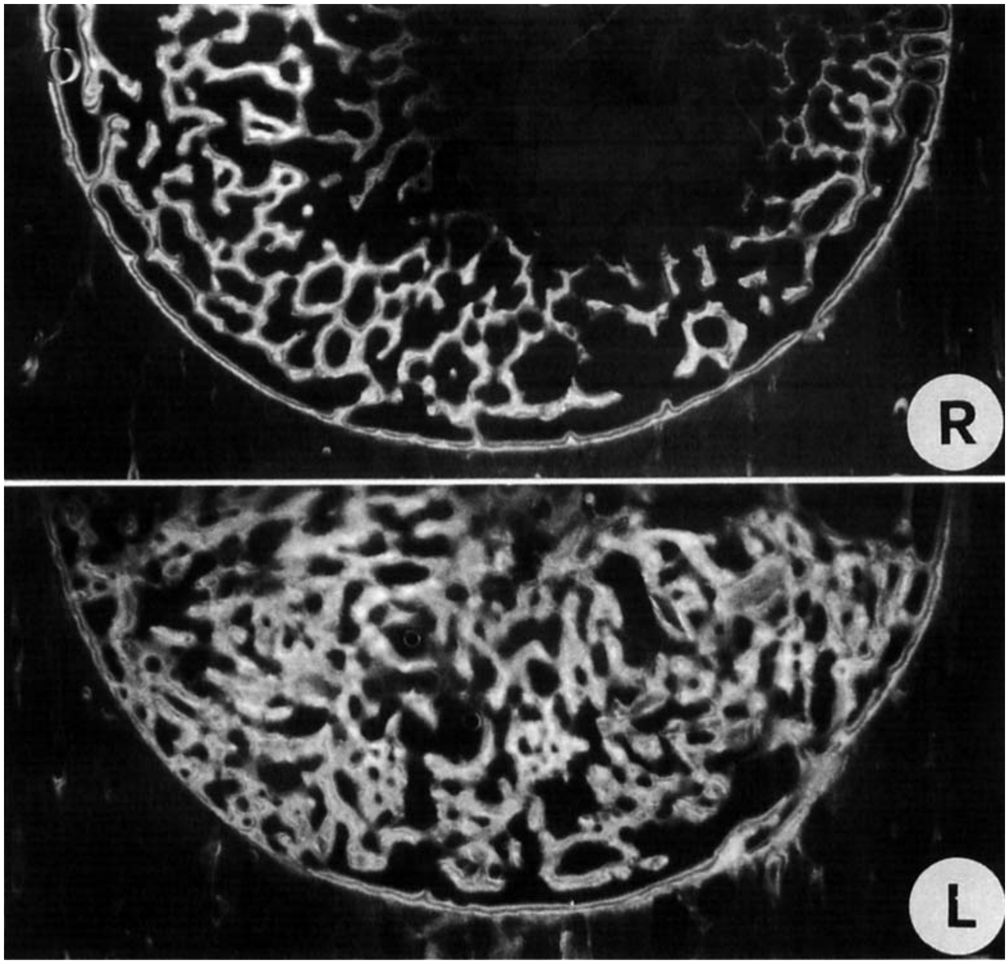

FIG. 3. Cross hemisections of the holes shown in Fig. 1 (photomicrographs under transmitted ultraviolet light; ×30). Two complete and coaxial rings of tetracycline are visible inside the layer of newly formed bone lining the surface of the holes.

(c) the area of the spaces not occupied by bone tissue—porosity (P). The relative amount of bone laid down during 30 days was expressed as a percentage ratio of NB/(NB + P). Statistical analyses were performed with the two-tailed paired Student *t* test.

The transverse hemisections of the holes were microphotographed under ultraviolet light (2 energizing filters [BG12] and 1 barrier filter [OG5]; Zeiss Ultraphot II, Oberkochen, Germany). On the photographic prints, made at a total enlargement of ×400, the distance between the two tetracycline labels was measured in the layer of newly formed compact bone that lines the internal surface of the holes; the spongy bone newly formed inside the holes was not taken into account in this investigation. This parameter, recorded every 140 μm, orthogonally with respect to the two labels (Fig. 4) and expressed in μm/day, was taken as an index of the MAR (nomenclature recommended by Parfitt et al. [20]). The statistical analyses were performed with a Student *t* test.

RESULTS

Light microscopic observation of the microradiographs of the mid-longitudinal sections of the holes (Fig. 1) showed, in both PEMF-treated and untreated sides, that the newly formed trabeculae originated not from the pre-existing bone but from a thin layer of newly deposited layer of bone lining the surface of the hole; moreover, the newly formed trabeculae were more densely arranged and more mineralized near the endosteal side than the periosteal surface. The stage of repair was always more advanced in the PEMF-treated holes than in the untreated holes.

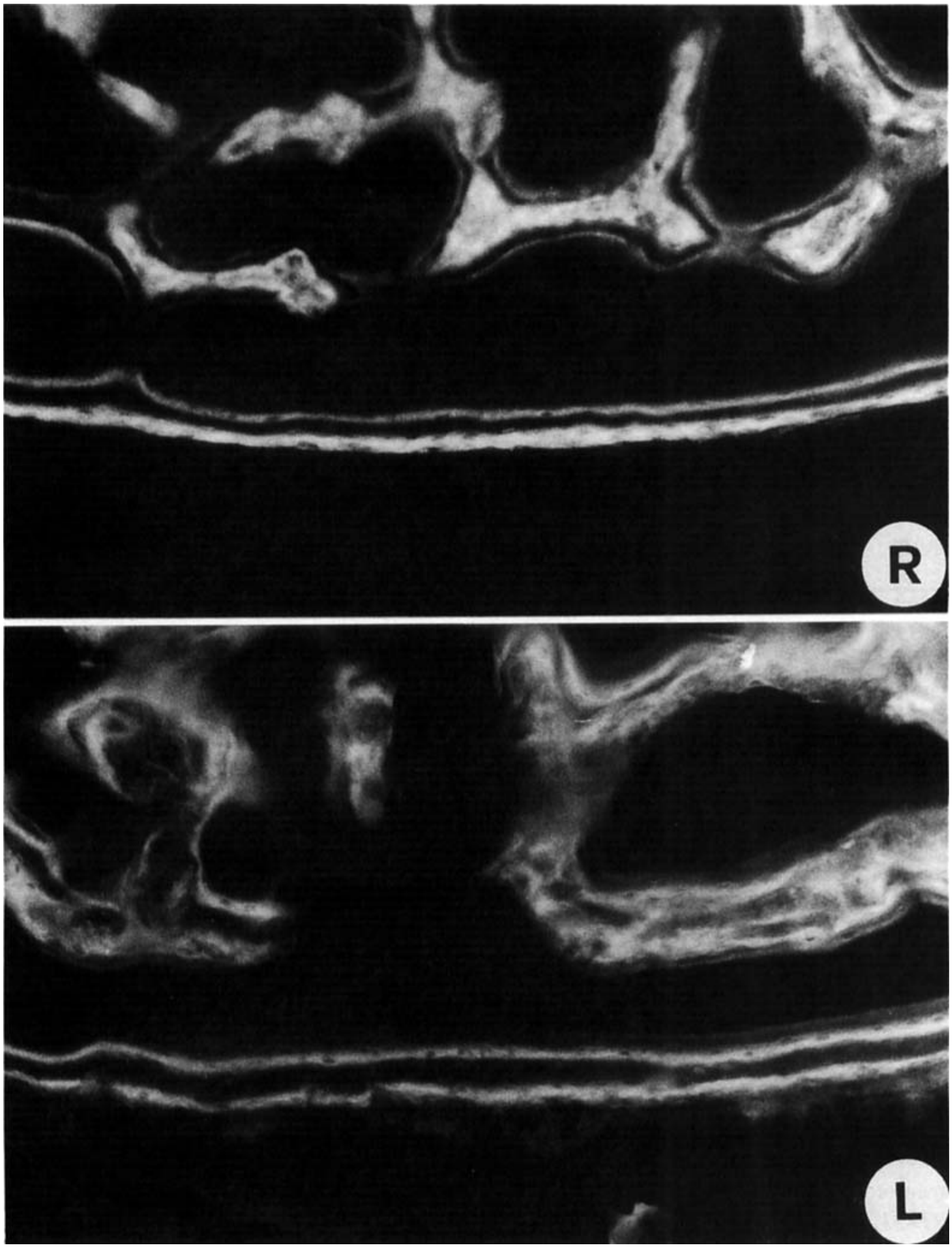

FIG. 4. Details at higher magnification (×200) of Fig. 2. The distance between the two labels is greater in the hole on the side treated with pulsed low-frequency electromagnetic fields (L).

The total amount of bone formed during 30 days in each hole is reported in Fig. 2. The values of the percentage ratio NB/(NB + P) were significantly higher ($p < 0.01$) in PEMF-treated holes than in the controls.

Microscopic observation under ultraviolet light of the transverse hemisections of the holes showed, in both PEMF-treated and untreated sides, two concentric and complete rings of tetracycline inside the layer of the newly formed bone lining the surface of the holes (Fig. 3). The interval between the two labels appeared to be greater in PEMF-treated holes than in the controls (Fig. 4). Also, the fluorescence of the newly formed trabeculae was, on the whole, more

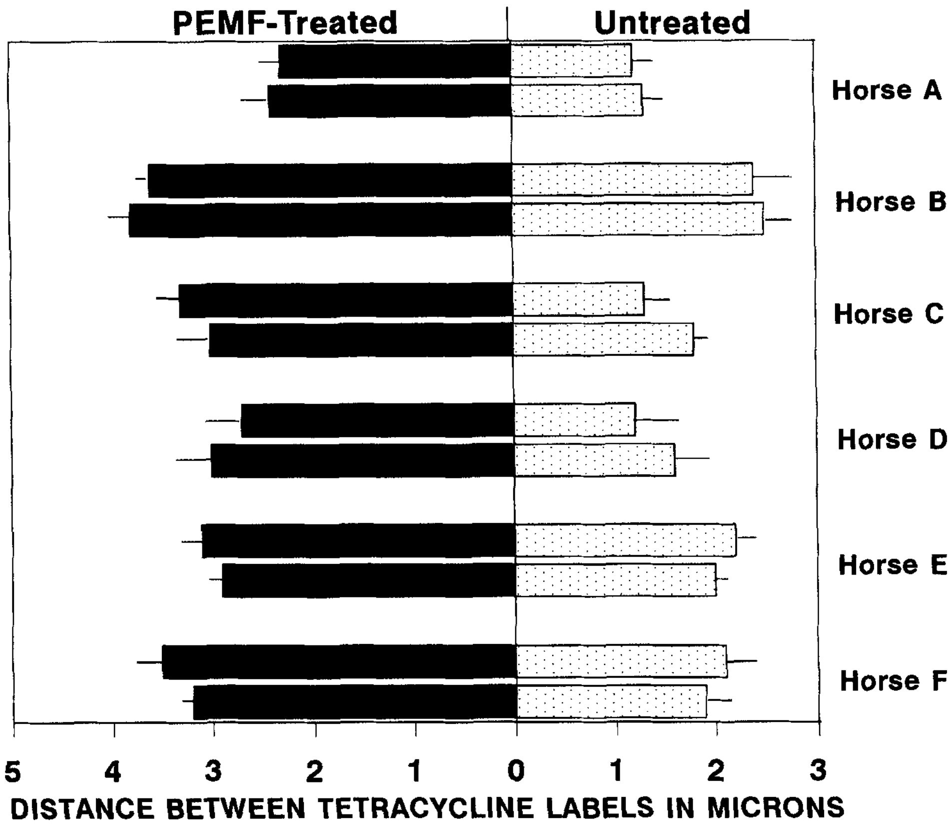

FIG. 5. Diagram of the mean values ± SE of the mineral apposition rate (MAR) during 30 days in two transcortical holes made at the same diaphyseal level in the homotypical metacarpals of six adult horses. Vertical axis: the two holes in the left metacarpals treated with pulsed low-frequency electromagnetic field (PEMF) (black bars) and the untreated metacarpals (right) (dotted bars) of each animal. Horizontal axis: the values for MAR, expressed in μm/day. Each bar represents the mean value calculated from 50 measurements (see text for explanation). The thickness and fluorescence of the newly formed trabeculae are greater in the PEMF-treated hole.

intense in PEMF-treated holes than in the untreated holes (Fig. 3).

The values for MAR are reported in Fig. 5; the distance between the two fluorescent labels appears to be significantly greater in the PEMF-treated holes than in the controls ($p < 0.0001$).

DISCUSSION

The effect of PEMFs on reparative osteogenesis has been investigated in several experimental and clinical models (1,3-5,7,8,15,21). It has been demonstrated, for instance, that PEMFs accelerate fracture healing (5) and promote the maturation of bone trabeculae (1). It also has been stressed that the positive effect on bone growth and repair seems to be related to specific PEMF signal configuration (1,5,21). This notwithstanding, the effect of PEMFs on the various stages of bone repair has yet to be defined.

For this reason, we attempted to elucidate whether PEMFs act during the osteogenic phase of transcortical hole repair. We restricted the analysis to the diaphyseal region, since only at this level does the reparative process appear to be constantly improved by PEMFs (12). In fact, at the metaphyseal level, the effect of PEMFs on transcortical hole repair was found to be inconsistent, being sometimes positive and sometimes negative; however, the symmetry in the rate of hole closure that we found between the left and right metacarpal bones in untreated horses (9,10) was never appreciable. Such a puzzling result in the metaphyses probably is related to the high rate of bone turnover in this region of long bones, as shown by the tetracycline labeling method (2,16,17). Indeed, it is plausible to suppose that the effect of PEMFs may be less regular, and thus less detectable, in spongy than in compact bone; that is, in regions where bone cell metabolism is higher.

The double tetracycline labeling technique was used in the present investigation because it appears to be the most precise method for evaluating the linear rate of accretion of the osteogenic surface. It must be noted, however, that tetracycline molecules, as well as those of other fluorochromes, become fixed to the growing bone crystals and not to the organic matrix secreted by osteoblasts (13,14,23). Therefore, they label not the newly formed osteoid surface but the mineralization front; consequently, the fluorochrome double-labeling technique enables measurement not of bone appositional growth rate (BAR) but of MAR. In this connection, it must be noted that the linear rate of osteoid matrix deposition does not differ significantly in a unit of time from the linear rate of bone mineralization when the rate of osteoid mineralization is not impaired (6,18,19,22). Therefore, in normal conditions, MAR may be considered a reliable index of BAR; that is, of osteoblast activity.

Briefly, since the data on MAR reported here may be referred to as osteoblast activity (our horses being normal as far as phosphocalcium metabolism is concerned), it may be concluded that PEMFs stimulate osteoblast activity during the healing process of transcortical holes, at least in diaphyseal compact bone. Whether or not PEMFs also affect the stages of bone healing preceding osteogenesis should be elucidated by further investigations.

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REFERENCES

1. Aaron RK, Ciombor DM, Jolly G: Stimulation of experimental endochondral ossification by low-energy pulsing electromagnetic fields. *J Bone Miner Res* 4:227-233, 1989
2. Amprino R, Marotti G: A topographic-quantitative study of bone formation and reconstruction. In: *Bone and Tooth, Proceedings of the First European Symposium Held at Somerville College, Oxford, April 1983*, pp 21-33. Ed by HJJ Blackwood. Oxford, Pergamon Press, 1964
3. Bassett CA, Pawluk RJ, Pilla AA: Augmentation of bone repair by inductively coupled electromagnetic fields. *Science* 184:575-577, 1974
4. 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
5. Bassett CAL, Valdes MG, Hernandez E: Modification of fracture repair with selected pulsing electromagnetic fields. *J Bone Joint Surg [Am]* 64:888-895, 1982
6. Bordier J, Marie P, Miravet L, Ryckewaert A, Rasmussen H: Morphological and morphometrical characteristics of the mineralization front: a vitamin D regulated sequence of the bone remodelling. In: *Bone Histomorphometry*, pp 335-354. Ed by PJ Meunier. Paris, Armour Montagu, 1977
7. 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: double-blind study. *Clin Orthop* 237:256-263, 1988
8. Bruce GK, Howlett CR, Huckstep RL: Effect of a static magnetic field on fracture healing in a rabbit radius: preliminary results. *Clin Orthop* 222:300-306, 1987
9. Cane V, Botti P, Remaggi F, Morotti 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
10. Cane 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
11. Cane 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
12. Cane V, Botti P, Farneti D, Soana S: Electromagnetic stimulation of bone repair: a histomorphometric study. *J Orthop Res* 9:908-917, 1991
13. Caswell AH, Hutchison JD: Selectivity of cation chelation to tetracyclines: evidence for special conformation of calcium chelate. *Biochem Biophys Res Commun* 43:625-630, 1971
14. Ibsen KH, Urist MR: The biochemistry and the physiology of the tetracyclines: with special reference to mineralized tissues. *Clin Orthop* 32:143-169, 1964
15. Kold SE, Hickman J, Melsen F: Preliminary study of quantitative aspects and the effect of pulsed electromagnetic field treatment on the incorporation of equine cancellous bone grafts. *Equine Vet J* 19:120-124, 1987
16. Marotti G: Quantitative studies on bone reconstruction. I. The reconstruction in homotypic shaft bones. *Acta Anat* 52:291-333, 1963
17. Marotti G: Map of bone formation rate values recorded throughout the skeleton of the dog. In: *Bone Morphometry*, pp 202-207. Ottawa, U of Ottawa Press, 1976
18. Milch RA, Rall DP, Tobie JE: Bone localization of the tetracyclines. *J Natl Cancer Inst* 19:87-93, 1957
19. Olah AJ: Quantitative relations between osteoblasts and osteoid in primary hyperparathyroidism, intestinal malabsorption and renal osteodystrophy. *Virchows Arch Abt A Path Anat* 358:301-308, 1973
20. Parfitt AM, Drezner MK, Glorieux FH, Kanis JA, Malluche H, Meunier PJ, Ott SM, Recker RR: Bone histomorphometry: standardization of nomenclature, symbols, and units: report of the ASBMR histomorphometry nomenclature committee. *J Bone Miner Res* 2:595-610, 1987
21. Pienkowski D, Pollack SR, Brighton CT, Griffith NJ: Comparison of asymmetrical and symmetrical pulse waveforms in electromagnetic stimulation. *J Orthop Res* 10:247-255, 1992
22. Takahashi H, Norimatsu H, Konno T, Inoue J, Yanagi K: Different patterns of tetracycline uptake in varying forms of rickets and osteomalacia. In: *Bone Histomorphometry, Third International Workshop, Sun Valley, May 28-June 2, 1980*, pp 87-93. Ed by WSS Jee and AM Parfitt. Levallois, Armour Montagu, 1981
23. Urist MR, Ibsen KH: Chemical reactivity of mineralized tissue with oxytetracycline. *Arch Pathol* 76:484-496, 1963