Pferdeheilkunde 10 (1994) 6 (November/Dezember) 413–428

Preliminary data on the effects of low-frequency pulsed electromagnetic fields on the physical resistance of bone

V. Canè, P. Botti[1], *Maria Antonietta Muglia, Francesca Remaggi* und *Silvana Soana*[2]

Department of Morphological Sciences and Forensic Medicine – Section of Human Anatomy, University of Modena, Italy;
[1] Department of Animal Pathology, University of Turin, Italy;
[2] Department of Veterinary Experimental Radiology, University of Parma, Italy.

Summary

The microhardness (HV) testing technique was applied to investigate the effects of low-frequency pulsed electromagnetic fields (PEMFs) on the degree of the mineralization of bone formed during the healing of transcortical holes. At the mid-diaphyseal level of the left and right metacarpal 3rd of 2 adult horses, 1 transcortical hole (4.5 mm diameter) was drilled. The hole in the left side was exposed for 30 days to PEMFs (28 Gauss peak amplitude, 1.3 msec rise time, 75 Hz repetition rate). The right contralateral untreated hole was taken as control. The results indicate:

(a) the amount of bone formed during 30 days is greater ($p<0.01$) in PEMF-treated holes than in contralateral untreated ones.

The HV values of new bone formed during 30 days

(b) are higher ($p<0.001$) in PEMF-treated holes than in the controls;

(c) in both PEMF-treated and untreated holes decrease from endosteum towards periosteum;

(d) are higher ($p<0.001$) in woven non lamellar bone than in lamellar bone.

These preliminary findings indicate that the increase in the physical resistance of reparative PEMF-treated bone seem to be related to the improvement of the healing process promoted by PEMFs. Nevertheless we cannot exclude that PEMFs interfere with mineral fraction and/or matrix components of bone.

keywords: Microhardness, Mineralization, Bone repair, PEMFs.

Vorläufige Ergebnisse über den Einsatz der niederfrequenten Elektromagnetfeld Therapie zur Bestimmung der physiologischen Festigkeit von Knochengewebe

Die Methode der Messung der Mikrohärte wurde eingesetzt, um den Effekt der niederfrequenten Elektromagnetfeld-Therpie zu bestimmen. Gemessen wurde der Grad der Knochen-Mineralisation von transkortialen Löchern. Bei 2 erwachsenen Pferden wurde in der Mitte der rechten und linken metacarpalenDiaphyse jeweils ein transkortikales Loch gebohrt. Das linke Loch wurde für 30 Tage den PEMFs ausgesetzt, das rechte kontralaterale Loch diente der Kontrolle.

Folgende Ergebnisse wurden erzielt:

a) bei mit PEMF-behandelten „Löchern" ist die gebildete Menge an Knochenmasse während 30 Tagen höher als bei unbehandelten Löchern.

b) Die Mikrohärte (HV-Werte) liegen bei den PEMF-behandelten Löchern weitaus höher als bei denen der Kontrolle.

c) Die HV Werte nehmen sowohl in den behandelten wie auch in den unbehandelten Löchern vom Endosteum zum Periosteum hin ab.

d) Die HV Werte liegen in den strukturierten nicht lamellären Knochen höher als in den lamellären.

Diese Erkenntnisse lassen die Annahme zu, daß die physiologische Festigkeit von Reparationsgewebe, welches PEMF-behandelt wurde, in engem Zusammenhang zu der verbesserten Heilung, welche durch PEMF hervorgerufen wurde, steht.

Es kann jedoch nicht ausgeschlossen werden, daß PEMF die Mineralbestandteile und / oder Komponenten der Matrix schädigend beeinflußt.

Schlüsselwörter: Mikrohärte, Mineralisation, Knochenheilung, PEMFs

Introduction

Our investigations carried out over the last decade demonstrate that low-frequency pulsed electromagnetic fields (PEMFs) exert a positive effect on the healing process of transcortical holes drilled in the diaphyseal region of long bones of 51 horses (*Botti* et al. 1992, *Canè* et al. 1991, *Canè* et al. 1992).

In order to verify whether PEMFs also interfere with the process of mineralization of bone matrix, we undertook a new series of experiments to evaluate the physical resistance of primary reparative bone in transcortical diaphyseal holes subjected to treatment with PEMFs. The microdurimetric technique was employed, which supplies absolute data that can be referred to the degree of mineralization of bone tissue; since microhardness is a function of the degree of mineralization, water content and orientation of collagen fibres *(Amprino* 1958, *Carlström* 1954, *Marotti* et al. 1972). As regard lamellar bone, lastnamed variable must be reconsidered in terms of density of texture of the collagen of bone matrix. Recent studies (*Marotti* 1990, *Marotti* and *Muglia* 1988, *Marotti* and *Muglia* 1989) have shown that the lamellar structure of bone does not depend on a different orientation of the collagan fibres (*Gebhardt* 1906) but, rather, represents a variety of bone tissue with woven fibres in which the texture of the collagen is alternately dense or loose. Thus if the values of the hydration and density of texture of bone matrix are constant, the variations in microhardness depend exclusively on variations in the degree of calcification.

Materials and Methods:

Two male horses, 8–10 years of age, were used. Under general anaesthesia by gas (Fluothane 1–2 % + O_2 30 % + N_2O 68–69 %), in accordance with our usual surgical methodology (*Canè* and *Botti* 1990) 1 transcortical hole was drilled at mid-diaphyseal level of the right and left 3rd metacarpus of each horse. As in our previous studies (*Canè* et al. 1991), the 3rd metacarpus of both sides were surrounded with a pair of Helmholtz coils (IGEA S.r.l., Carpi, Italy). Only the coils placed round the left metacarpus were activated for 30 days by a pulse generator (IGEA-stimulator, IGEA S.r.l., Carpi, Italy). The magnetic field signal was characterized by 28 Gauss peak amplitude, 1.3 msec rise time, 75 Hz repetition rate. The value of the electric field induced in a standard probe ranges between 3.25 ± 0.25 mV on the left; the electric field induced in the right antimer, controlled by means of a standard probe rated a sensitivity level of 0.01 mV, was not detectable. The animals were put down at 30 days after application of the coils.

The bone fragments containing the holes were freed of soft tissues by an aqueous solution of 3 % NaOCl and were embedded, not decalcified, in methylmethacrylate (BDH Italia S.r.l, Milan, Italy). From each hole a midlongitudinal section was taken (Leitz 1600 Microtome, Leitz, Wetzlar, Germany). The sections were microradiographed by contact on photographic plate with support in poliester (ILFORD EM, Ilford Ltd, Mobberley, Cheshire, U.K)

A computerized image analyzer (TESAK, Florence, Italy) was used to calculate on the microradiographs of the midlongitudinal sections of the holes the following:

(a) total area of hole;

(b) area occupied by the bone contained in it (NB);

(c) area of the spaces not occupied by bone tissue (porosity P).

The relative amount of bone laid down during 30 days is expressed by the percentage ratio of NB/(NB+P). Statistical analysis was performed by two-tailed paired Student's t-test.

The rate of mineralization of the bone laid down during 30 days was deduced from the degree of microhardness (HV) obtained by means of the microdurimetric method (*Favia* 1976, *Marotti* et al. 1972). The microdurimeter (Leitz-Durimet, Leitz, Wetzlar, Germany) was used to assay the physical resistance of woven bone, in the lamellar (L) and nonlamellar (NL) forms, the bone layer laid down on the surface of the holes (peripheral layer), the trabeculae adjacent to it (peripheral spongy bone) and the trabeculae axially situated (axial spongy bone). Tests of these architectures were performed at the 1/3 proximal (endosteal zone), 1/3 intermediate (intermediate zone) and 1/3 distal (periosteal zone) of the holes. On the bone surface a square-base pyramidal diamond indenter (Vickers pyramid with 136° angles) was applied with a load of 15 g. HV expressed in Kg/mm^2, is calculated by the formula $HV = 1854.4\ L/D^2$ (L = load in g, D = mean length, expressed in µm, of the diagonals of the print measured by optical micrometer). Statistical analysis of the results was performed by Student's t-test.

Scanning electron microscope analysis (Philips SEM-515, Philips S.p.A., Milan, Italy) of the texture of the collagen fibres of the new bone formed during 30 days was performed on the same sections treated with HCl 0,1N for 90 sec, trypsine 80 U/ml (Trypsin 1:250 Porcine, GIBCO Ltd, Paisley, U.K.); pH 7,4 for 41 h at 37 °C and metallized with gold-palladium (Sputter Coater SCD-004, Balzers S.p.A., Milan, Italy).

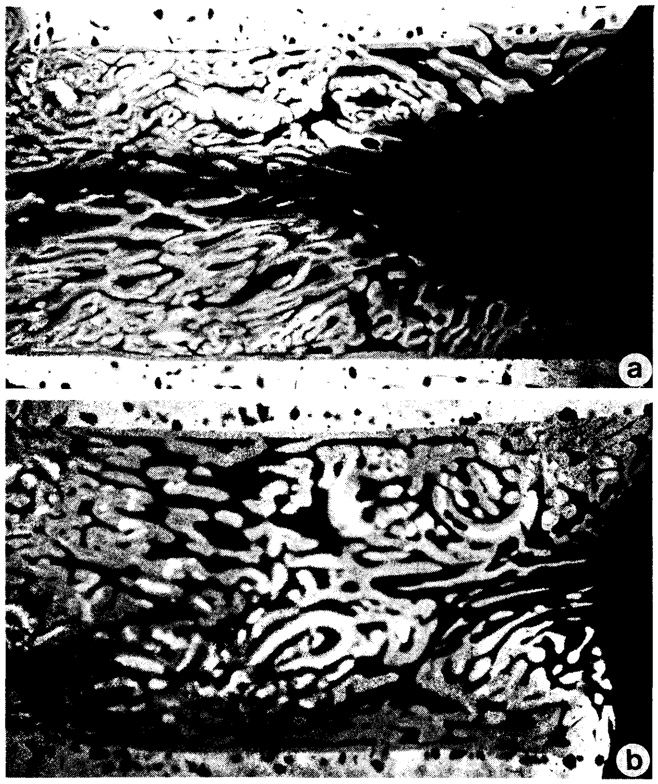

Fig. 1: Microradiographs of mid-longitudinal section of a transcortical hole drilled at mid-diaphyseal level in right (a) untreated metacarpus 3rd and in left (b) PEMF-treated. (Microphotographs in transmitted ordinary light; x21). In both holes repair proceeds from the endosteal side (left) to the periosteal side. Note also that the amount of newly formed bone is greater in PEMF-treated hole.

Results

In both PEMF-treated and untreated holes the new bone has a spongy architecture that originates from a thin bony layer connected with the preexisting compact bone by means of a sort of cementing line (Fig. 1). Within it may be distinguished thick trabeculae (tubular spongy bone) circumscribing cylinder-like cavities that evolve into primary osteons, and thin trabeculae (laminar spongy bone) delimiting wide vascular spaces (Fig. 1). Scanning electron microscope analysis clearly shows that the spongy bone is structured in woven-fibred bone tissue in the non-lamellar and lamellar forms (Fig. 2) that coexist only in the tubular trabeculae; whereas the laminar trabeculae are made up of woven-fibred non-lamellar form only. The absence of cementing lines shows that the new formed bone has the structural features of a primary bone.

In the control holes the tubular and laminar trabeculae, situated respectively in the peripheral and axial portions, are present in the endosteal and intermediate zones; the periosteal zone lacks laminar trabeculae. In PEMF-treated holes tubular trabeculae are present in all three zones, whereas laminar trabeculae are found only in the periosteal zone.

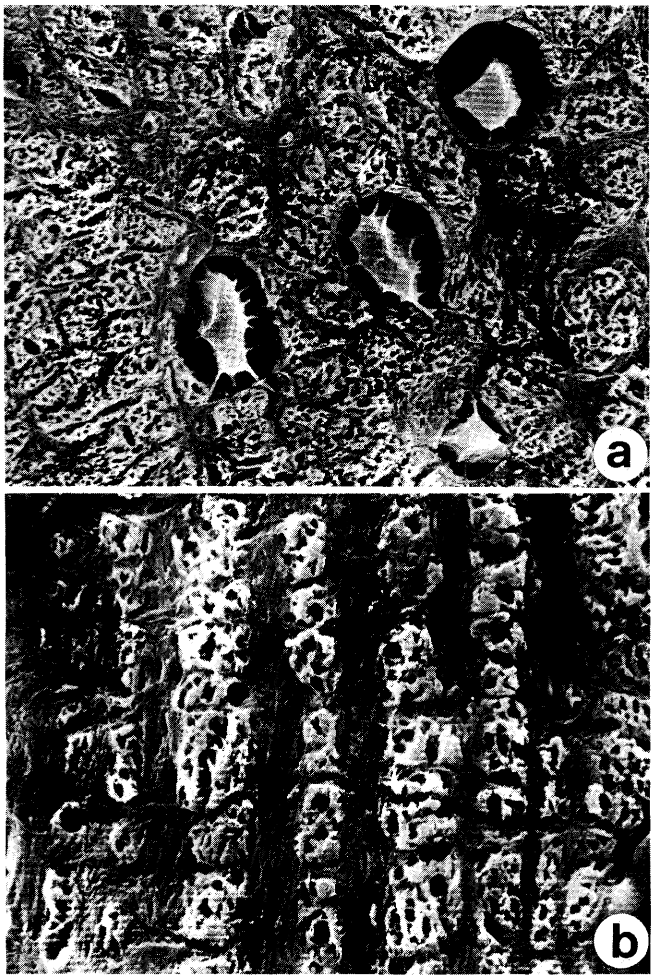

Fig. 2: Microphotographs with scanning electron microscope (x3100) showing the structure of newly formed bone in the holes during 30 days. (a) woven-fibred non-lamellar bone tissue; (b) woven-fibred lamellar bone tissue where dense lamellae alternating with loose lamellae can be seen.

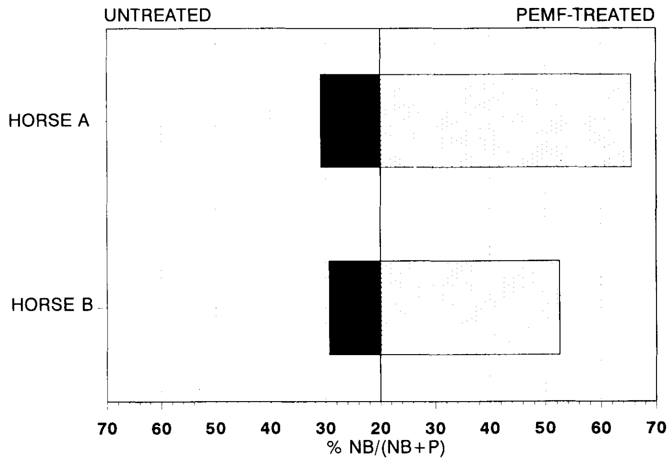

Fig. 3: Diagram of the amount of bone laid down through 30 days in the middiaphyseal transcortical holes made in the homotypical metacarpi 3rd of two horses. On the vertical axis: the two holes in the untreated right metacarpus (black columns) and PEMF-treated left metacarpus (dotted columns) of each animal. On the horizontal axis: values of %NB/(NB+P), where NB is the newly formed bone and P the porosity.

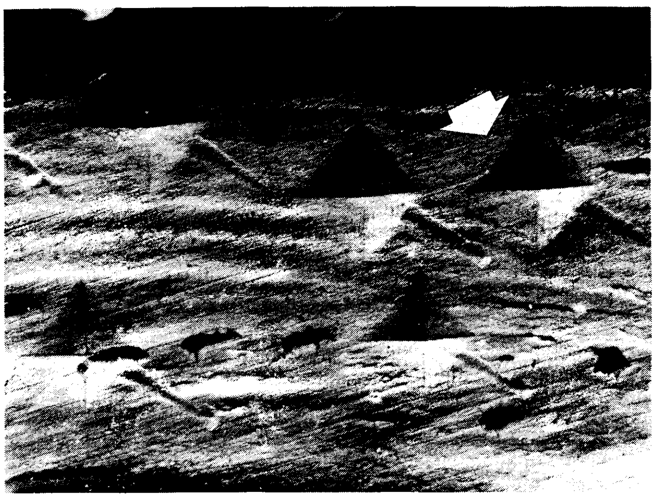

Fig. 4: Microphotograph with scanning electron microscope of part of the bony trabecula (x482). In both PEMF-treated bone and untreated one the prints of the pyramid of Vickers (constant load of 15 g) are greater in the lamellar (arrow) than in the non-lamellar bone, since the latter was laid down earlier.

In both treated and untreated holes the lamellar structure gradually diminishes on going from the endosteal to the periosteal zone. In particular, this structure is either wholly absent, or present in quantities too small to be assayed, in the periosteal zone of the peripheral bony layer, of the peripheral spongy bone and of the axial spongy bone.

Comparison of treated with untreated holes show that the former repair was quicker. The values of %NB/(NB+P) are higher ($p<0.01$) in PEMF-treated holes (Fig. 3).

The HV values of the bone structures in the treated holes are higher ($p<0.001$) than those of the homologous untreated structures (Fig. 4, 6). In both treated and untreated holes, the HV values decrease on going from the endosteum towards the periosteum and are higher in non-lamellar than in lamellar bone (Fig. 5, 6), in fact the imprints are larger in the latter.

Discussion

In agreement with our previous investigation (*Canè* et al. 1991), the results of the structural analysis shows that PEMFs do not seem to alter the modality of repair of transcortical holes made in the diaphyseal region of horse metacarpus 3rd. Indeed, both in the PEMF-treated holes and in untreated ones repair proceeds by laying down of spongy bone, from the endosteal extremity to the periosteal extremity, with trabeculae that do not originate directly from preexisting compact bone but rather from a thin layer of new bone formed on the surface of the holes. In its general features, the repair process of this type of lesion follows the same stages that characterize normal periosteal growth of the diaphyses; in other words, the new bone at first has a spongy architecture with laminar trabeculae that gradually evolve into tubular trabeculae and finally into primary osteons. Thus the different architecture and microscopic

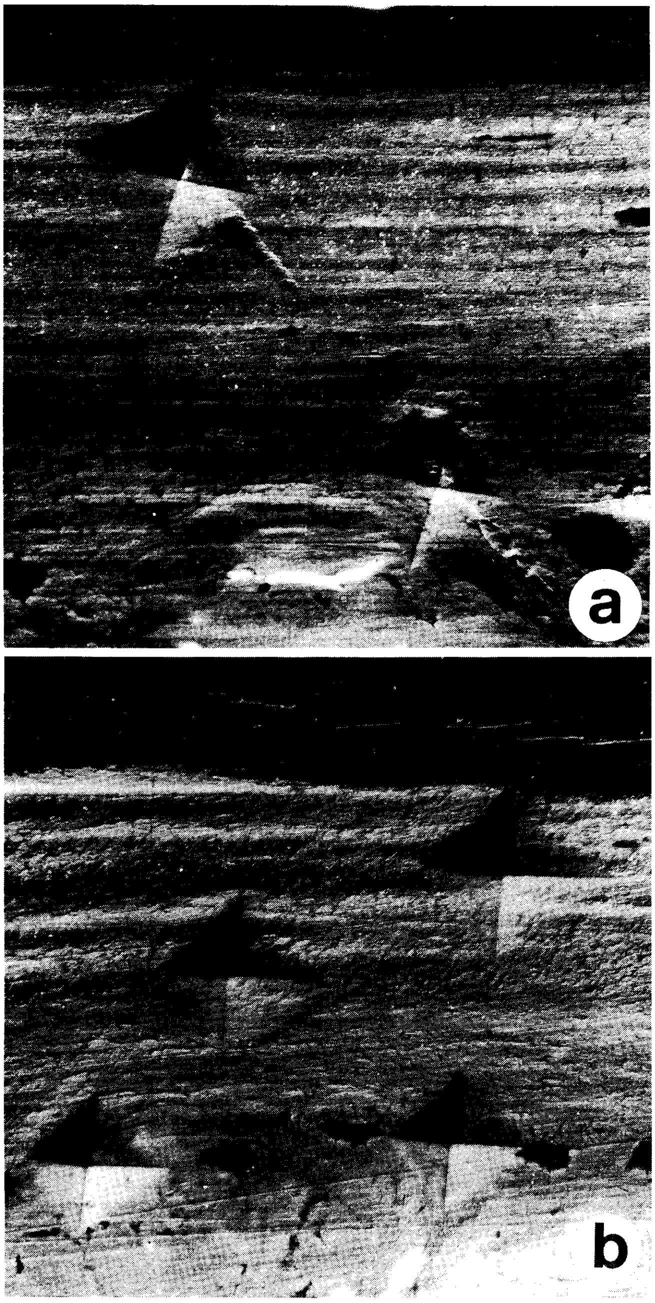

Fig. 5: Microphotographs with scanning electron microscope of part of the peripheral bony layer of the endosteal zone in a control hole (a) and (b) a PEMF-treated hole (x1300). The prints of the pyramid of Vickers (constant load 15 g) are on average greater in the control hole than in the PEMF-treated one.

structure found in the holes between the endosteal side and periosteal side represent successive states of the above-mentioned repair process (*Canè* and *Botti* 1990).

The findings regarding the amount of new bone formed agree with those of our previous studies in indicating a noteworthy increase in quantity of new bone formed in the treated holes (*Canè* et al. 1991, *Canè* et al. 1992), and show that PEMFs hasten repair of this type of lesion at diaphyseal level.

With regard to the evaluation of the effect of PEMFs on the mineralization rate of bone, it should be mentioned that in this experiment we were not able to employ the double tetracycline labelling technique (*Marotti* 1963), which would have enabled comparison of the microhardness of bone laid down at the same time in the treated and untreated holes. This must be reserved for a further study. In the present one we have attempted to prove whether a correlation between physical resistance of the bone and the extent of repair, in the treated and untreated holes exists, with reference to the entire duration of the experiment.

The finding that the HV values of newly laid down bone in the PEMF-treated holes are greater than those of the untreated bone should, in our opinion, mainly be ascribed to the fact that bone began to be laid down earlier in the treated holes.

In both treated and untreated holes, the HV values decrease, showing a regular trend in the non-lamellar bone and very irregular one in the lamellar, going from the endosteal extremity to the periosteal one. This datum emphasizes that in this type of lesion repair commences near the endocortical side of the hole (*Canè* and *Botti* 1990) and proves that the microhardness of the bone is closely related to the mineralization rate and, perhaps, with the density of the collagen of the bone matrix. The bone tissue that is first laid down evidently has a higher mineralization rate as compared to the bone laid down subsequently. In addition, this bone tissue is characterized by homogeneous matrix, in the sense that it shows no sharp differences in density. The bone tissue that is formed after the non-lamellar one is obviously characterized by a lower mineralization rate and by a structure presenting superimposed densely woven layers of bone matrix alternating with loosely woven ones (*Marotti* 1990, *Marotti* and *Muglia* 1988, *Marotti* and *Muglia* 1989). This last aspect is translated into the constitution of the dense lamella, where the bone matrix is densely aggregated, and the loose lamella, where the components of the matrix are more dissipated. These two types of lamella correspond respectively to the transverse and longitudinal lamellae in the classic model of *Gebhardt* (1906).

Therefore, although a possible interference of the PEMFs also on the mineral fraction and/or the components of the bone matrix cannot be ruled out, these preliminary results enable us to posit that the greater physical resistance found in bone newly laid down in the transcortical diaphyseal holes treated with PEMFs is the natural consequence of acceleration brought about by the PEMFs in the repair process of this type of lesion (*Botti* et al. 1992, *Canè* et al. 1991, *Canè* et al. 1992).

Acknowledgment:

We particularly would like to acknowledge the guidance, support, and counsel of Prof. Gastone Marotti, Director of the Institute of Human Anatomy in the University of Modena. The authors are especially indebted to IGEA S.r.l. (Carpi, Italy) for supplying the stimulating device. This investigation was supported in part by a "grant 40%" from the Italian Ministry of University and Scientific and Technological Research.

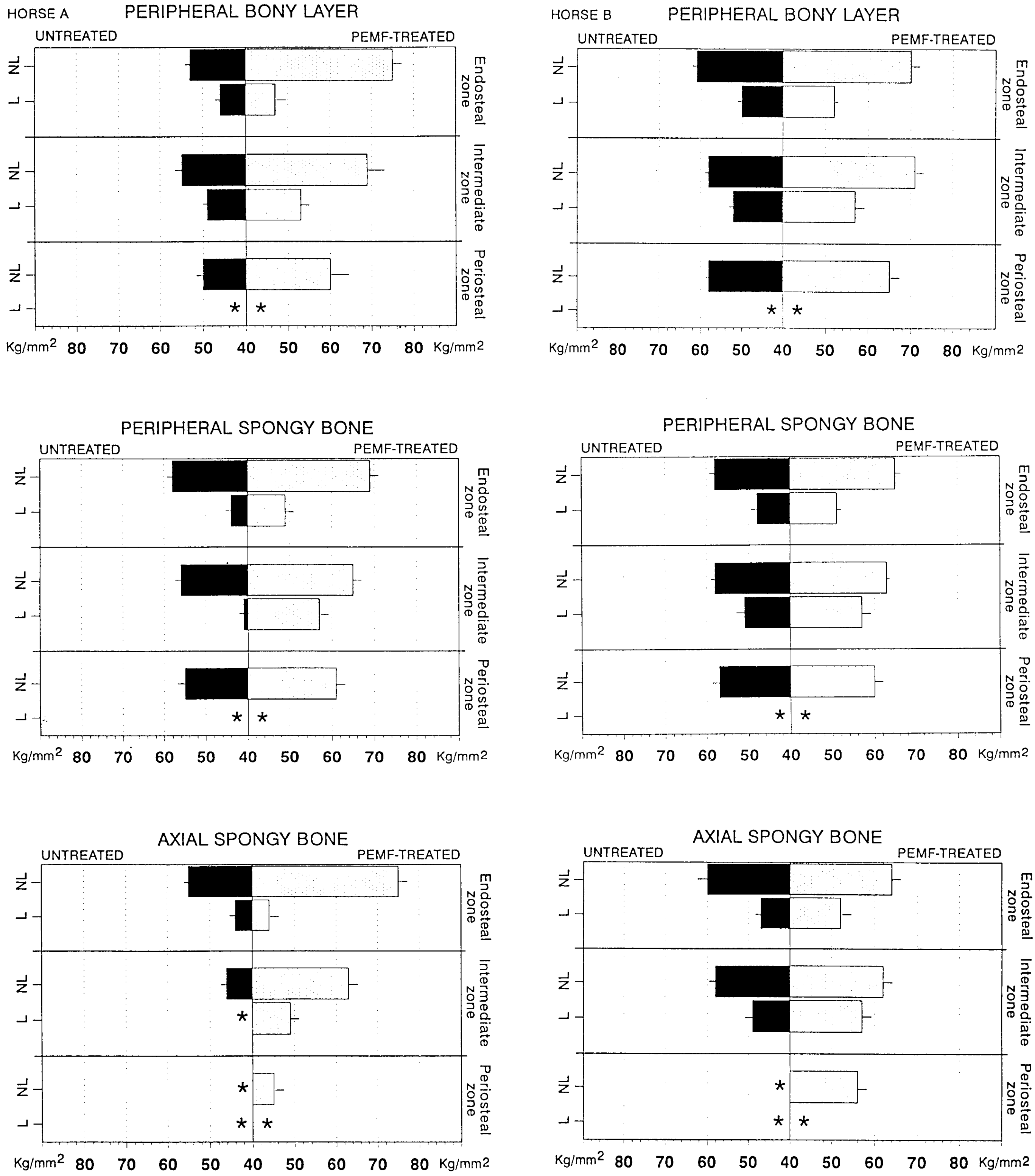

Fig. 6: Diagrams of the degree of the microhardness performed by means of the microdurimetric method. Mean and Standard Deviation, indicated on the columns, of the mineralization rate in bone laid down in transcortical holes treated with PEMFs (dotted columns) and untreated holes (black columns) drilled at mid-diaphyseal level of the metacarpus 3rd in two adult horses. On the left vertical axis is indicated the non-lamellar (NL) and lamellar (L) structure of the bony layer formed around the surface of the holes (peripheral layer), of the bony trabeculae situated near the layer (peripheral spongy bone) and of the bony trabeculae present on the longitudinal axis of the holes (axial spongy bone). On the right vertical axis are indicated the three portions of the hole. In these portions the microhardness of the aforementioned structures was tested. On the horizontal axis are indicated the microhardness values (HV) expressed in Kg/mm^2.

The absence of the corresponding structure is indicated by * .

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Prof. Valerio Canè

Dipartimento di Scienze Morfologiche e Medico Legali
Sezione di Anatomia umana
Via Del Pozo, 71 (Policlinico)
I - 41100 MODENA (MO) - ITALY
Telephone: ++39 ++59-374818
Telefax: ++39 ++59-372695