On direct currents and bone formation in demineralized bone transplants

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The effect of direct current on the remineralization of a bone defect was studied in the rabbit. Bone defects in the radius of both forelegs were grafted with demineralized autologous bone. On both sides platinum electrodes were placed around the graft, and one side was connected to a power source delivering 20 μA constant current during the experimental period of 28 days. The remineralization was evaluated 14 and 28 days after operation by scintigraphy and roentgenography, planimetry included. At 28 days after operation this evaluation was supplemented by autoradiography. Roentgenographically, there was no difference between the two sides. At 14 days after operation scintigraphy demonstrated a minor delay in bone formation at the electrostimulated side. Between 14 and 28 days a significant increase in activity was noticed. On both sides, autoradiograms showed areas without uptake around the wires. It was concluded that direct currents of the studied magnitude have a negative influence on the primary bone induction process but also that it seems to influence the mineralization positively later in the bone-forming process. □ *Autoradiography; demineralized bone; electrical current; osteogenesis; radiography*

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The first attempts to stimulate bone healing by electricity date to the middle of the 19th century (1). The hypothesis that electrically generated energy could stimulate the growth and healing processes in bone tissue evoked particular interest when Yasuda (2) published a now classical paper demonstrating that bone had stress-generated piezoelectric properties and that direct current of 1 μA over 3-week period could produce callus around a cathode placed in bone. Corresponding results of in vivo and in vitro studies have been reported by, for example, Basset (1), Basset & Becker (3), Shamos & Lavine (4), Basset (5), Herbst (6), and ElMessiery (7). Many clinical studies have reported success with this method in up to 80% of cases of nonunion and pseudoarthrosis (8–11).

Basically, two different types of systems are at present used clinically. Stimulation is either carried out by direct currents in an invasive form by implantable stimulators (9, 10) or by pulsing electromagnetic fields in a noninvasive system (11). Basset (11) claimed that pulsing electromagnetic fields have an effect on clinical situations that far exceeds that of constant direct currents. The main reason is that pulsing systems are noninvasive and that they do not give rise to the anodic toxic effects often observed in experiments with direct currents. To minimize the negative effects around the anodes of implantable devices, pulsing current has been introduced (12, 13). According to Dwyer & Wickham (14) and Paterson et al. (9), there is no definite difference in the osteogeneic capacity between direct and pulsing current methods.

Autologous demineralized bone in a radial bone defect has previously been shown to have better osteogeneic properties than autologous bone containing mineral (15).

Since electricity has clinically been claimed to accelerate bone formation in bone defects, it was considered of interest to study the effect of electric stimulation on the mineralization process in a bone defect grafted with autologous demineralized bone. Since rabbits were used as experimental animal, it was decided to use an implantable

system for electrostimulation generating direct currents.

Materials and methods

General outline

Nineteen adult rabbits with an average body weight of 3.5 kg were used. The operation was performed with the rabbits under anesthesia consisting of intramuscular injection of Hypnorm Vet® (Leo, Helsingborg, Sweden) and intravenous injection of pentobarbital (Mebumal®, ACO, Solna, Sweden) supplemented with local anesthesia (Xylocaine®, 0.5%, Astra, Södertälje, Sweden).

Initially, a 12-mm piece of the radius was resected bilaterally and demineralized in 0.6 N HCl for 24 h. After a thorough rinsing in saline these pieces were kept at +4°C for 2 days and, in a second operation, reimplanted in the defects with triple-helix electrodes (cathodes) surrounding the demineralized pieces of bone. The materials and methods used at these operations have been described in detail by Wittbjer et al. (16). The implanted electric stimulators generated a constant current of 20 μA during the experimental period of 28 days.

At 14 days after operation the remineralization process was evaluated by roentgenography and scintigraphy. At 28 days the rabbits were killed, and the same evaluation was performed, supplemented with autoradiography (17).

Electrostimulator

The implanted electrostimulator was equipped with six Berec® PX/RM 400 1.35-V mercury cells. It had a field effect transistor in series with a resistor controlling the circuit delivering 20 μA constant current (18). Two platinum electrodes with a length of 40 mm and a diameter of 0.5 mm were connected to the stimulator through polyethylene-insulated stainless steel wires.

The electrostimulating device was surgically implanted into the back of the rabbit and its electrodes tunneled subcutaneously to the radial defect. The anode was placed over the muscle fascia on the medial side of the humerus at a distance of approximately 40 mm from the defect area. The helical cathode with the inserted bone matrix was placed in the radial defect and fixed with sutures. The wounds were closed in anatomical planes. The procedures described were performed randomly on either the right or the left forelegs of the rabbits. The platinum wire and bone matrix were placed in the same manner on the experimental as on the contralateral control side, but without connection to a stimulator. An outline of the experimental arrangements is given in Fig. 1. After the rabbits had been killed, the stimulators were tested to verify that constant current levels were generated.

Roentgenography

The bones were examined with a dental X-ray unit (Oralix 65, N.V. Philips, Eindhoven, The Netherlands), using Kodak Occlusal Ultra-Speed D films and an exposure time of 0.4 sec. During the roentgenographic and scintigraphic examination 14 days after operation the rabbit was anesthetized with Hypnorm Vet. Twenty-eight days after the operation the right and left foreleg specimens were placed on the same X-ray film. The resulting roentgenograms were placed in an X-ray film enlarger/viewer (Realist Inc., Photographic Products Division, Wisconsin, USA) with an optic magnification of 10:1. The display was covered with transparent paper, on which the outlines of the bone defect and the remineralized tissue inside the bone defect were drawn. The remineralized area was then estimated with a planimeter (Los Angeles Scientific Instruments Co., Los Angeles, Calif., USA). The extent of the remineralized area was expressed in percentage of the area of the initial bone defect. The extent of mineralization was judged to be equal on the two sides when the difference was 3% or less.

Scintigraphy

^{99m}Tc-DPD, a technetium-99m-labeled 3,3-diphosphono-1,2-propanedicarboxylic

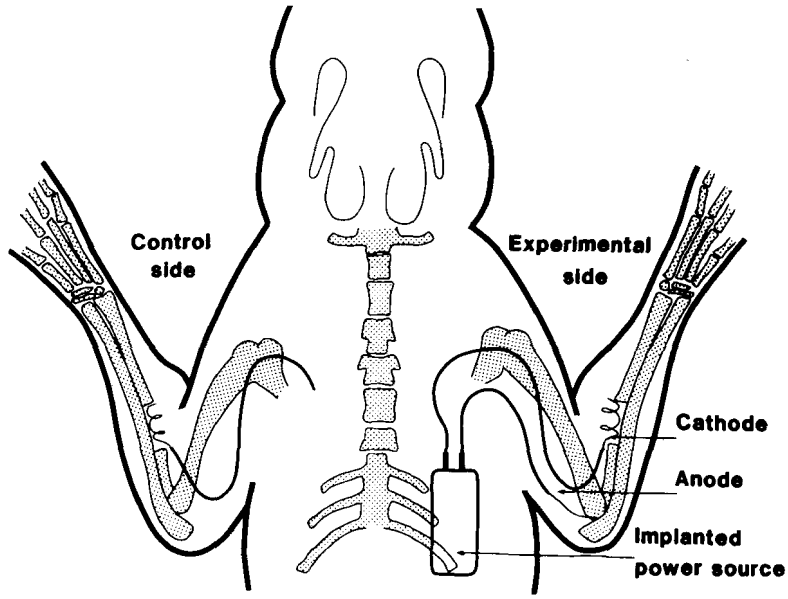

Fig. 1. Implanted power source with electrodes leading to the radial defects. On one side the electrode is connected to the power source.

acid tetrasodium salt (Behringwerke AG, Marburg, FRG) was injected intravenously 3 h before the animals were killed. The injected solution contained an activity of approximately 350 MBq. By means of the methods described by Wittbjer et al. (16), the gamma radiation was registered, and mean counts were calculated in regions mapped out covering the defect area. Values relative to a control area outside the defect were then calculated. The mean values in every region (Figs. 5 and 6) and the relative values in the center, proximal, and distal part of the defect on the experimental side (active cathode) and on the control side (passive cathode) were also calculated (Table 2, Fig. 7).

Autoradiography

After scintigraphy and roentgenography and 4 h after injection of ^{99m}Tc-DPD the foreleg specimens from seven randomly chosen animals were freeze-sectioned. Autoradiography was then performed by apposition of the sections and remaining frozen block against Structurix D7 films (Agfa-Gevaert, Antwerpen, Belgium). Sections and films were kept under refrigeration during exposure and then developed and fixed. The technique for freeze-sectioning and autoradiography has been described in detail by Ullberg (19) and the autoradiographic technique for ^{99m}Tc-labeled sections by Rohlin & Hammerström (20) The modifications of this technique for the experimental design used in this study have been described by Wittbjer et al. (17). Selected sections were stained with hematoxylin and eosin and then mounted.

Results

In 8 rabbits the electrodes were disconnected from the power cells or displaced from the experimental areas during the experimental period, leaving 11 rabbits for evaluation.

Roentgenography

At the outsides of the radii at the sites of resection, a triangular radiopacity representing callus was seen (Figs. 2, 3A, and 4A).

Fourteen days after the operation the rabbits were examined alive, and therefore the image quality sometimes was not quite satisfactory owing to movements during the

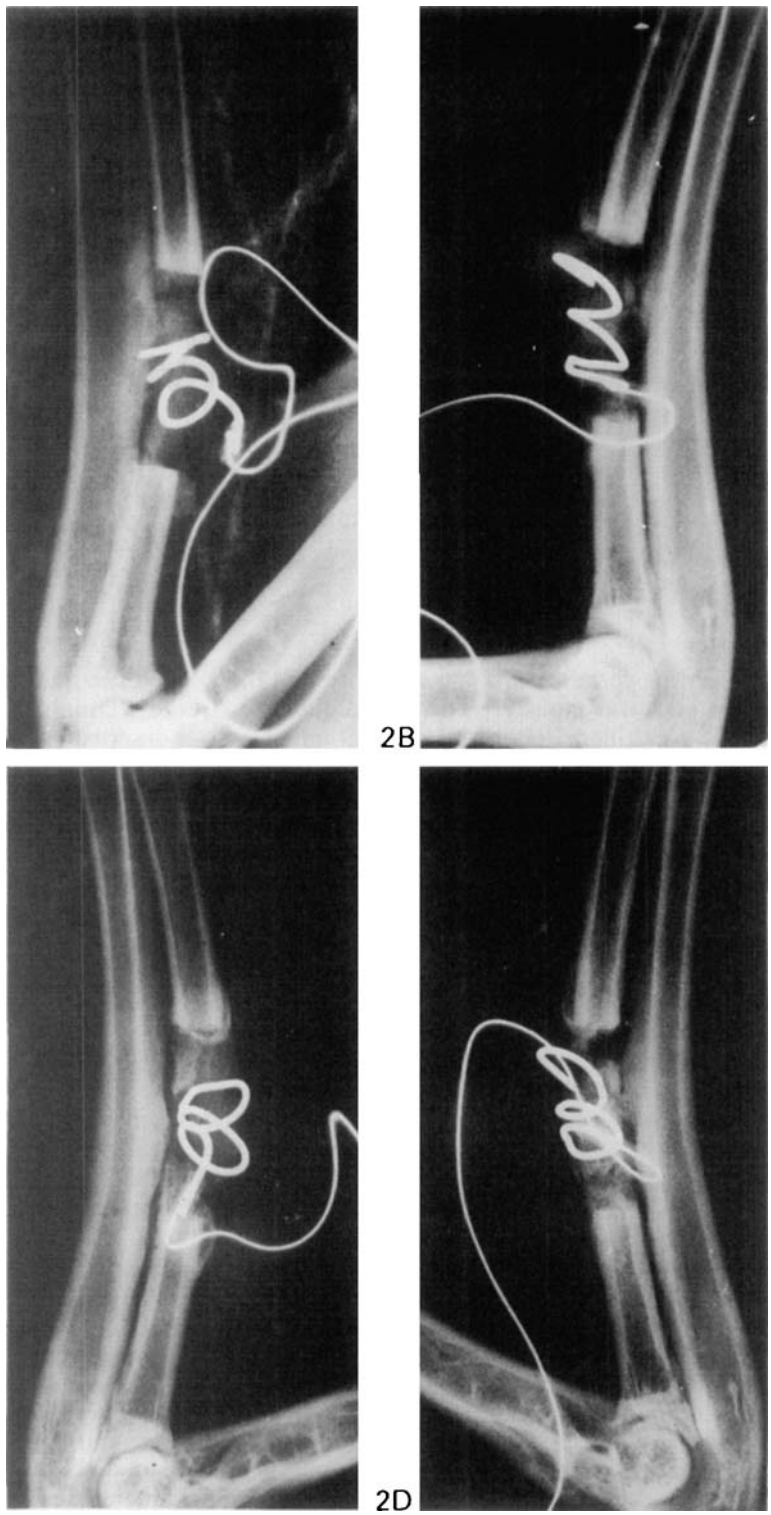

Fig. 2. Roentgenograms of the right and left radius and ulna from an adult rabbit 14 days (A, B) and 28 days (C, D) after operation. Left side with active electrode and right side with passive electrode. (Magnification, ×2.) Fig. 2A, B. There are scattered areas of mineralized tissue in the bone defect. The extent of mineralized tissue is about the same on the two sides. Fig. 2C, D. The extent of mineralized tissue has increased considerably compared to 2A and B. On the side with the active electrode there is somewhat more mineralized tissue. On both sides areas without mineral are seen. At the sites of resection there is callus formation on the outside of the radius. In the defect area periosteal thickening is seen on the ulna.

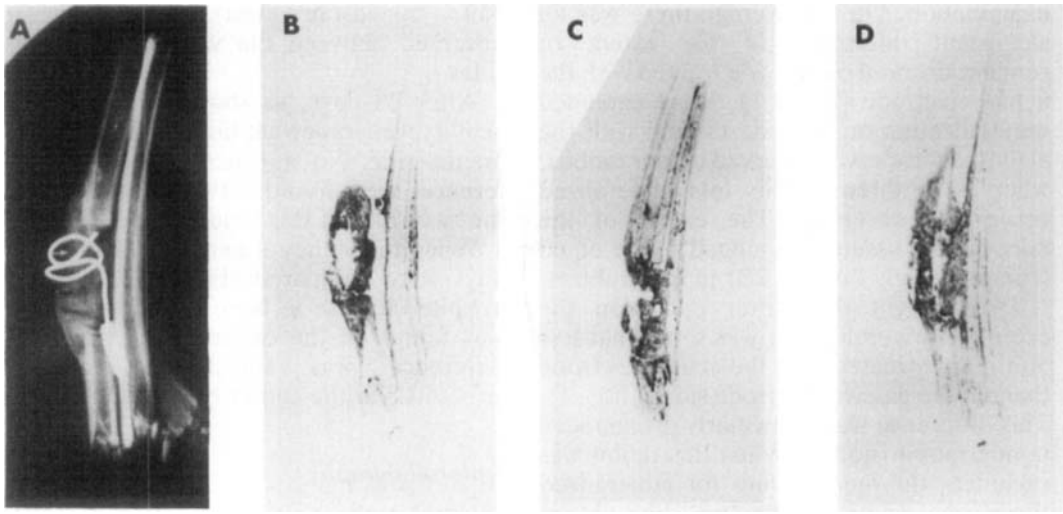

Fig. 3. Roentgenograms (A) and autoradiograms at three sagittal levels (B, C, D) of the radius and ulna from an adult rabbit 28 days after operation. The inserted electrode was active. (Magnification, ×2.) Fig. 3A. Almost all tissue inside the bone defect is mineralized. There are, however, small radiolucent areas inside the newly mineralized tissue (arrows). Fig. 3B, C, D. Uptake of ^{99m}Tc 4 h after intravenous injection of ^{99m}Tc-labeled DPD. The extent of uptake varies with the level of sectioning. Centrally, an area without uptake is observed. Compared with Fig. 3A the extent of mineral deposition is smaller in these three sections.

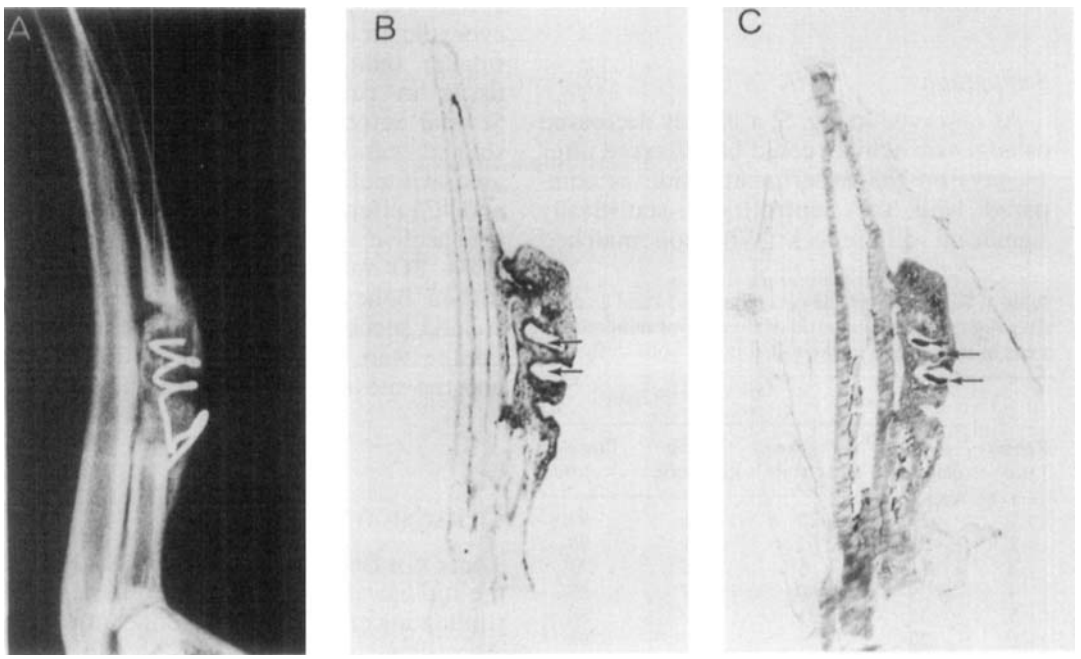

Fig. 4. Radius and ulna in an adult rabbit 28 days after operation. The wire was not connected to the electrostimulator. (Magnification, ×2.) Fig. 4A. Roentgenogram shows that almost the whole bone defect is filled with mineralized tissue. Close to the wire there are radiolucent areas. Fig. 4B. Autoradiogram shows the uptake of ^{99m}Tc 4 h after intravenous injection of ^{99m}Tc-labeled DPD: the uptake is seen in a large area of the defect. Well-defined areas without uptake lay centrally (arrows). Fig. 4C. Section corresponding to Fig. 4B. Around the wires (arrows) well-defined areas without tissue are seen.

examination. On the average there was no significant difference in the extent of remineralization on the side treated with the active electrode (Table 1). More extended mineralization on the side treated with the active electrode was observed in four rabbits, whereas in three rabbits less mineralized tissue was observed. The extent of the mineralized tissue was judged to be equal (compare Figs. 2A and 2B) in four rabbits.

Twenty-eight days after operation the extent of mineralization was somewhat less on the side treated with the active electrode than on the passive electrode side (Table 1). This difference was particularly pronounced in one rabbit (no. 6). When this rabbit was excluded, the mean values for mineralized tissue was equal for the two sides. More extended mineral deposition was seen on the side treated with the active electrode in three rabbits (compare Figs. 2C and 2D) and on the side treated with the passive electrode in four rabbits. In the remaining four rabbits the extent of mineralized tissue was judged to be equal on the two sides.

Scintigraphy

As observed in Fig. 5, a slightly decreased osteogeneic activity could be observed after 14 days on the experimental side as compared with the control. No statistically significant differences (Wilcoxon matched pairs signed-rank test) were, however, observed between the values for the two sides.

After 28 days, as shown in Fig. 6, the activity was somewhat higher on the experimental side. No statistically significant differences were found between the results of the two sides at this time either.

When the values obtained after 14 and 28 days were compared (Fig. 7, Table 2), a definite increase in bone formation activity was found on the experimental side. This difference was statistically significant ($p < 0.02$) in the center of the defect.

Table 1. Roentgenographic examination 14 and 28 days after operation: planimetric evaluation of mineralized areas in percentage of the radial initial bone defect

	14 days		28 days	
Rabbit no.	Active electrode	Passive electrode	Active electrode	Passive electrode
1	19	20	66	94
2	57	62	99	99
3	21	5	75	73
4	39	40	87	57
5	31	35	43	58
6	20	29	23	98
7	28	30	100	89
8	70	31	100	100
9	94	91	100	100
10	26	24	51	35
11	45	32	85	98
Mean	41	36	75	82

Autoradiography

Uptake of ^{99m}Tc ranged from extensive (Figs. 3C and 4C) to small areas of the bone defect (Figs. 3B, 3D, and 4B). The extent of uptake varied with the level of sectioning in the same specimen (Figs. 3B, 3C, and 3D) at some levels similar to the areas with newly mineralized tissue revealed by roentgenography (compare Figs. 3A and 3C). However, the areas with uptake were most often smaller than the areas with mineralized tissue in corresponding roentgenograms. Several autoradiograms and corresponding stained sections showed small well-defined areas without uptake or tissue (Figs. 3D, 4B, and 4C) often located around the electrodes irrespective of active or passive electrode (Figs. 3D and 4B). No remnants of unresorbed bone matrix could be seen in the stained sections. Small, thin areas without uptake were located between the implant and the sites of resection in some sections.

Discussion

There is a lack of detailed knowledge about the biological processes behind the electrostimulating enhancement of bone formation. According to Davidovich & Shanfeld (21), the stimulus provided by bioelectrical currents may involve an initial depolarization of cell membranes. Metabolically based differences in electrical potential frequently exist across layers of cells (22), and since

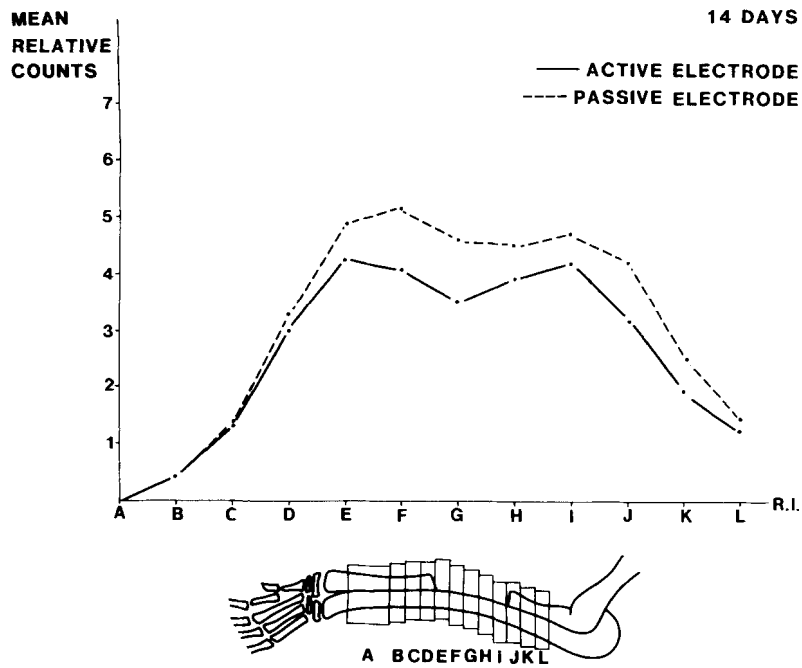

Fig. 5. Scintigraphic examination. Mean relative activity to the control area (A) in regions of interest (RI) 14 days after operation on the experimental side (active electrode) and control side (passive electrode). A value of 1 has been subtracted from the figures compared with those given in Table 2).

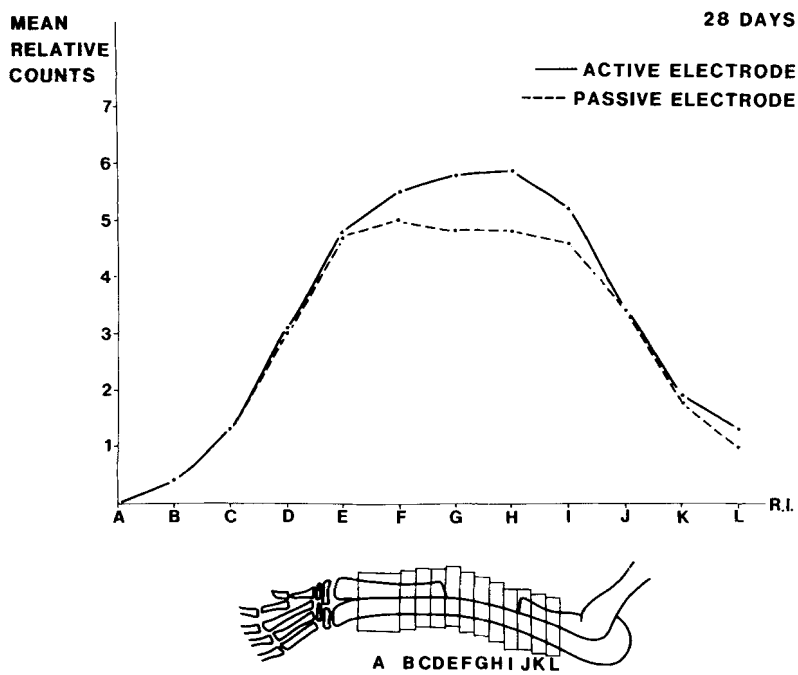

Fig. 6. Scintigraphic examination. Mean relative activity to the control area (A) in regions of interest (RI) 28 days after operation on the experimental side (active electrode) and control side (passive electrode). A value of 1 has been subtracted from the figures compared with those given in Table 2).

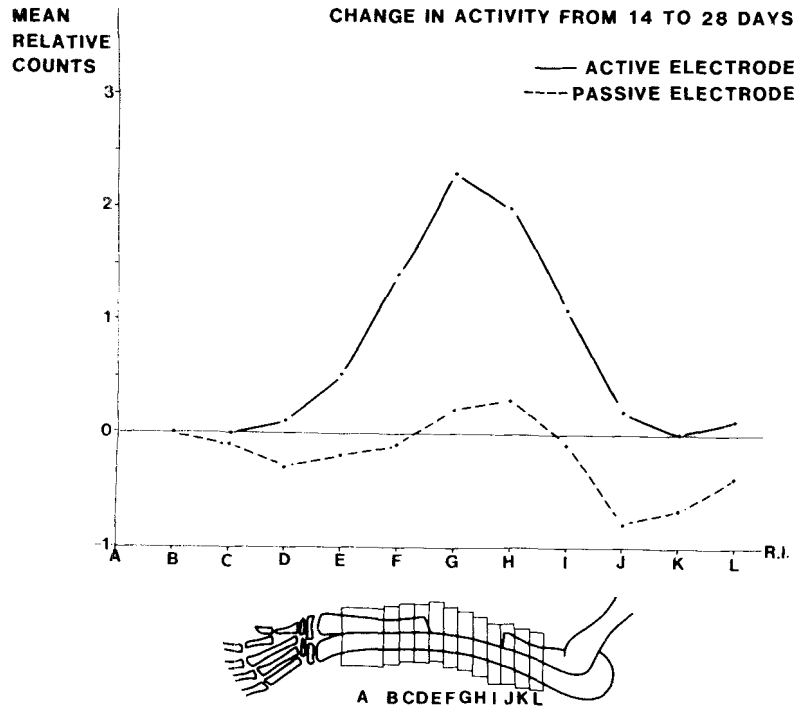

Fig. 7. Scintigraphic examination. The difference in mean relative activity to the control area (A) in regions of interest (RI) between 14 and 28 days after operation on the experimental side (active electrode).

ionic transport across bone membranes results in differences in electrical potential, such differences could perhaps promote osteogenesis. Bearing in mind the relatively low current densities used in this study, it is, however, reasonable to assume that the currents had a comparatively minor influence on the transport of ions in the examined tissues. Considering this, Justus & Luft (23) suggested a mechanochemical hypothesis regulating extracellular calcium influencing the osteoblastic activity. Such events could not be evaluated by the methods of the present study, which reveals the presence of mineral. However, for clinical applications it is important to evaluate the quality and quantity of bone tissue formed.

It was considered important to select for the cathode a metal or metal alloy having electrochemical properties with minimal toxic reactions. Platinum was selected because it has been shown to be a suitable electrode material and more osteogeneic than, for example, titanium and stainless steel (24). This is probably because of its favorable electrochemical properties and ability to catalyze the oxygen reactions believed to be of importance in bone formation (25). Friedenberg et al. (26) examined five different electrode positions in relation to fracture location. Best results were obtained with the cathode in the fracture and the anode in bone tissue or soft tissue at some distance from the fracture. A similar arrangement was used in the present study.

Both in clinical studies and in experimental ones various amounts of direct current have been used. At current levels about 10 μA anodic necrosis has been observed, and at levels above 100 μA necrosis around the cathode was also observed (27). As an adequate current level for osteogenesis was considered to be within the range of 5–20 μA, with an optimum effect at 20 μA (27–29), this current level, corresponding to an approximate current density of 0.3 μA/mm^2, was used in the present experiments. Furthermore, the current level of 20 μA is also used for clinical treatment of pseudoarthroses (10, 18).

After 2 weeks the scintigraphic evaluation

Table 2. Scintigraphic examination 14 and 28 days after operation: relative activity of the radial defects at the center and at the sites of resection (proximal, distal)

Rabbit no.†	14 days						28 days					
	Active electrode			Passive electrode			Active electrode			Passive electrode		
	Proximal	Center	Distal	Proximal	Center	Distal	Proximal	Center	Distal	Proximal	Center	Distal
1	5.5	2.9	4.9	*	*	*	8.4	6.1	5.3	4.1	3.6	6.4
2	3.5	2.3	4.1	6.4	5.7	7.4	8.9	13.0	5.9	8.1	10.9	7.9
3	7.6	5.9	8.2	6.1	3.8	6.0	8.2	7.7	7.8	5.7	5.2	4.9
4	5.7	5.2	7.5	8.3	5.8	8.7	5.1	5.6	4.7	4.7	5.3	6.0
5	4.1	2.0	4.1	5.2	3.3	6.5	3.5	2.1	3.5	3.2	2.0	4.8
6	4.5	2.6	4.0	8.0	3.7	5.9	5.7	3.5	5.9	8.9	7.4	7.5
7	7.9	6.9	8.4	9.8	9.9	10.8	4.8	6.7	5.5	5.5	5.8	5.6
8	6.0	7.3	6.1	4.5	5.1	3.4	5.7	7.6	6.4	*	*	*
9	4.8	5.0	4.3	5.6	6.5	3.8	7.7	8.6	6.7	5.5	6.5	6.0
10	*	*	*	3.9	4.4	3.8	4.2	3.4	4.2	4.3	3.8	4.8
11	3.9	3.6	5.0	4.8	5.6	4.3	*	*	*	*	*	*
Mean	5.4		5.7	6.3	5.4	6.1	6.2		5.6	5.6	5.6	6.0
		4.4				$p < 0.02$**		6.4				

* Data lost in computer files.

** *P* value in accordance with Wilcoxon's matched pairs signed-ranks test.

† Rabbit no. corresponds to number in Table 1.

showed a slight negative osteogeneic effect from electric currents in this study. After 4 weeks, however, the influence evaluated by scintigraphy had turned into a slight positive one, confirming results presented by Petersson et al. (18). The differences between the two observation periods were statistically significant for the side with electric current. According to Rodan et al. (30, 31), small electrical currents provide a 'trigger stimulus' or threshold that initiates a sequence of cellular events. These involve the proliferation of stem cells and their subsequent differentiation into an osteogeneic cell line. At the beginning of the observation period in the present study matrix, but few cells, was available for stimulation. According to Spadaro (24) and Basset (11), it is conceivable that optimal field strength differs at each stage of healing, since different cells are involved at different times. It is most likely that the electric current disturbed that initial inductive influence from the implanted matrix which the implant previously was shown to have in this experimental design (16). Compared with these findings, less mineralization and bone-forming activity to some extent confirms the statement. Later in the bone-forming process, when an adequate amount of osteoblasts are present, there is a significant increase in bone-forming activity compared with 14 days after operation. These findings can be due to the earlier noticed delay in the osteogenesis but also to a possible appropriate field strength influencing the metabolic activity of the cells. Such a reaction is supported by the findings of Herbst et al. (29) that electrostimulated bone formation in tissue cultures was associated with an increase in the metabolic activity rather than an increase in the quantity of cells.

In pseudoarthroses the osteogeneic activity of electricity is believed to be associated either with a stimulation of the collagen matrix to act as a receiver for hydroxyapatite (32) or with an alteration of the proteoglycan-rich cartilage, creating conditions for mineralization (33). However, the collagen structure in the fibrous or fibrocartilaginous tissues bridging a pseudoarthrosis has no inductive capacity of its own (34) and thereby differs from the matrix of implants used in the present study.

In this study well-defined areas around both wires without uptake were seen in autoradiograms, and lack of tissue close to the wires was seen in the stained sections. This could be interpreted as a reaction to the metal and is in contrast to the findings of Spadaro (24) demonstrating that platinum stimulates osteogenesis even without current. Since this appeared on both sides independently of electrical stimulation, the level of current could not have caused the reaction. A continuous movement of the wires, however, might have obstructed the aggregation of cells, resulting in the well-defined borderlines of the areas.

Summing up, the observed lack of definite electrostimulating effects on bone formation in demineralized bone does not indicate that electricity per se has no influence on osteogenesis. It rather indicates that electrical stimulation might be more effective in a later phase of bone formation, similar to the effect described in pseudoarthroses and fibrous tissue bridging a nonhealed bone defect or effective in stimulation of existing bone-forming cells.

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