THE RESPONSE OF THE FEMUR TO TRAUMA, A FOREIGN BODY, AND A DIRECT ELECTRICAL CURRENT IN MICE†

The idea that electricity may be used by orthopedists to repair injured and diseased bone and by orthodontists to help straighten crooked teeth is being popularized. It started when investigators found that piezoelectrical-type potentials are generated in stressed bone. Areas of bone under compression develop negative potentials, whereas areas under tension develop positive potentials. It has long been known that when a living bone is forcibly bent it will become thicker on the compressed concave side and thinner on the tensed convex side. Negative potentials that occur where the bone becomes thicker and positive potentials that occur where the bone becomes thinner may or may not be causative factors in the remodeling of the bone. Although the generation of piezoelectrical potentials in stressed bone is not the same as the passage of an exogeneous direct current through unstressed bone, Lavine, et al. reported significantly more new bone formation in drilled rabbit femurs receiving a direct current than in non-electrically treated drilled femurs. Bassett, et al. reported massive production of endosteal bone around every cathode of insulated battery implants in one femur each of 12 dogs. The electrodes projected into the medullary cavity of the femur and a direct current of 10 $\mu$A or 100 $\mu$A was maintained for 14-21 days. They noted an increased cellularity at the cathode during bone formation and felt it was the result of mitoses. Other investigators performed similar experiments using the femur in rabbits and obtained equivocal or negative results. O'Connor, et al. used detailed directions supplied by Bassett to duplicate his experiment on the dog femur. They found that the cathode did not always have the most bone formation around it. In two dogs it had the least. We performed an experiment on mice that was similar to those above; however, some modifications were made that we felt were improvements in experimental design.

MATERIALS AND METHODS

Adult inbred mice (Brown-belt) were arranged into three groups. In the first group of 12 males and 12 females (Group I) the shaft of the right femur was exposed. Two

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holes were made 5 mm. apart through the shaft using a stainless steel tubular drill (.006" diameter) rotated at a high speed. A wire electrode (.005" diameter) was passed through each proximal and distal hole to protrude 1 mm. beyond the femur on the opposite side. In half the number of males and females the electrodes were platinum. In the other half they were stainless steel. A Teflon washer was placed over the protruding part that was then bent at a right angle to hold the electrode in place. The other end of each of the two electrodes in the femur was attached to a battery pack situated under the skin of the animal's back. The battery pack consisted of a Mallory Rm 400 RT2 battery (1.35 volt) in series with an eight watt, 13,500 ohm resistor. The pack was embedded in dental cement (Fastcure®), after which it was coated with epoxy (Chemgrip®) followed by a coating of Silastic Medical Adhesive. Each pack delivered 100 µA of direct current when tested at the beginning and at the end of the experimental period. In 6 males and 6 females the positive electrode was inserted into the hole made in the proximal end of the femoral shaft and the negative electrode into the hole made in the distal end. In the other 6 males and 6 females the positions of the positive and negative electrodes were reversed. The wires and packs did not interfere with the normal locomotor activity of the mice. The animals of Group I were killed two weeks after they received the battery packs. The electrodes were removed from each femoral shaft that was then excised, preserved, decalcified, embedded in paraffin, sectioned serially at 7 µ, and stained with hematoxylin and eosin.

The second group of 12 males and 12 females (Group II) received exactly the same operative treatment as those of Group I except that they received a dummy pack lacking a battery and a resistor.

The third group (Group III) consisted of 14 females with a live pack (negative electrode inserted proximally in the shaft) and 14 females with a dummy pack. In half the number of females with live or dummy packs the electrodes were platinum. In the other half they were stainless steel. Two mice with a live pack and two with a dummy pack were killed daily during a 7-day period beginning the first day after surgery. Each mouse received an injection of 50 µgm colchicine six hours before it was killed to permanently halt any mitotic cells at metaphase. The right femoral shafts of Groups II and III were prepared for microscopic study in the same manner as those of Group I.

RESULTS AND CONCLUSIONS

The tissue changes were similar in the sections from the mice of Group III that bore live packs when compared with those that bore dummy packs. The sections of the mice killed at 24 hours after surgery showed the extent of the tissue destruction made by the drill (Fig. 1A). At the site of entry of the drill into the femoral shaft the cut edges of the periosteum formed a collar around the wire. The drill made a clean-cut hole through the compact bone except near its exit at the endosteal surface where bony lamellae were bent into the medullary cavity. Small broken pieces of bone were scattered among tissue debris around the wire at the endosteal surface. Tissue debris completely surrounded the wire within the medullary cavity. Mesenchymal cells were scattered throughout this debris and immediately adjacent to it were numerous large multinucleated phagocytes that contained vacuolated
debris within their cytoplasm (Fig. 1A). The drill made a clean-cut hole through the compact bone at the opposite side of the femoral shaft except near its exit at the periosteal surface where bony lamellae, including the attached periosteum, were bent away from the shaft. Small pieces of bone were scattered among tissue debris that surrounded the wire at the site of exit of the drill.

A steady progression of similar tissue changes was seen in the sectioned shafts of the mice of Group III that bore live or dummy packs before being killed at daily intervals from 1 to 7 days. The changes were essentially the same at both the platinum and stainless steel wires in both the proximal and distal ends of the shafts in each mouse. At the end of one week hyaline cartilage was found beneath the periosteum at both the site of entry and exit of the wire from the femoral shaft (Fig. 1B). The amount of cartilage at the site of exit was extensive and was in direct contact with trabecular bone that constituted a bony callus. Also, trabecular bone formed a callus at the site where the wire passed from the compact bone into the medullary cavity (Fig. 1B). However, no callus cartilage was found at this endosteal site in the femoral shafts from any of the mice. The matrix of the compact bone of the shaft immediately surrounding the wire gradually changed so that by one week it resembled fibrous connective tissue. This fibrous tissue was continuous through the medullary cavity as a sleeve around the wire (Fig. 1B). In mice killed on the fifth to the seventh day the fibrous tissue surrounding the wire adjacent to the bony callus, where the drill originally passed from the compact bone into the medullary cavity, transformed into bone (Fig. 1B). The lamellae of this new bone were circumferentially arranged around the wire. At one week, numerous vascular sinusoids of the medullary cavity were located close to the wire and multinucleated phagocytes were still present in this region.

The femurs from the control mice of Group II that bore dummy packs for two weeks had a small bony callus beneath the periosteum at the site of entry of the wire and a large one beneath the periosteum at the site of exit of the wire at both the proximal and distal ends of the shaft (Fig. 1C). No callus cartilage was present at these sites. The bony callus at the endosteal site, where the wire passed from the shaft into the medullary cavity, was so large it nearly bridged the cavity (Fig. 1C). The hole that housed the wire was surrounded throughout its extent by compact new bone with circumferential lamellae. The matrix of the new bone was directly continuous with that of the original compact bone of the shaft and with the trabecular bone of the calluses. Small marrow cavities were situated along the line of junction of the matrices of the new bone and the original compact bone of the shaft (Fig. 1C).
The microscopic changes in the shafts from 8 male and 8 female mice of Group I that bore live battery packs for two weeks were the same as those in the shafts from the control mice of Group II that bore dummy packs for two weeks. In the remaining 4 male and 4 female mice of Group I the changes were less advanced, being similar to those found in the shafts from the mice of Group III killed at one week. The electrodes in three of these eight mice were platinum. In each mouse of Group I the extent of the changes was essentially the same at the site of the positive and negative electrodes. Also, it made no difference whether the positive and negative electrodes were located at proximal or distal positions in the shaft or whether they were platinum or stainless steel.

We examined every cell of every tissue section of the entire experiment with a high power 100X oil immersion lens and found only one cell undergoing mitosis. It was a mesenchymal cell in the medullary cavity from a mouse of Group III that was killed at five days after surgery. Therefore, we conclude that the numerous cells in every femur that formed the new bone surrounding the wire in the medullary cavity must have come from the pool of mesenchymal cells of the marrow known as connective tissue progenitor cells. These cells have been shown by the autoradiographic technique to be able to modulate into either chondroblasts and/or osteoblasts in response to local environmental changes.11-13 The results of this study contribute to the presently published experimental data that fail to justify the use of a direct electrical current as a clinical application in an attempt to repair injured and diseased bone or to help straighten crooked teeth.

SUMMARY

Bone formed around wires conducting a direct electrical current completely through femurs of mice for two weeks never exceeded or was less than the amount of bone formed around non-conducting control wires. Cartilage appeared and disappeared during bony callus formation around all of the wires at the femoral periosteal surfaces but not at the endosteal surfaces. Connective tissue progenitor cells in the femoral marrow modulated into osteoblasts and formed bone within the medullary cavity around the full extent of all of the non-conducting control wires but not around the full extent of all of the wires conducting a current.

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Fig. 1. Reconstructions of serial sections through the mouse femur where a non-conducting control wire was situated. (A) From a mouse killed 24 hours after the wire was inserted. (B) From a mouse killed one week after the wire was inserted. (C) From a mouse killed two weeks after the wire was inserted. Abbrev.: (P) periosteum; (CBS) compact bone of femoral shaft; (HMC) hematopoietic and mesenchymal marrow cells; (MP) multinucleated phagocytes; (S) vascular sinusoid; (H) hole where the wire was situated. Adjacent arrow indicates the direction the hole was drilled for the insertion of the wire; (D) cellular and extracellular debris; (CC) callus cartilage; (T) trabecular bone; (F) dense fibrous tissue; (CBW) compact bone that surrounded the wire; (CB) callus bone.
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