

Regeneration after physeal distraction of the radius in sheep

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In 18 sheep, gradual physeal distraction of the distal radius was performed by external fixation. Separation of the physis from the metaphysis occurred in all the sheep. Prior to bone formation, collagen fibers were organized according to the direction of the distraction, and lamellar bone was formed if the collagen bundles were lineally organized. In the area where the collagen was disorganized, and also near the periosteum, woven bone was first formed, which was then remodeled into lamellar bone. In some sheep separate groups of chondrocytes were isolated in the metaphyseal area after the distraction. In 2 animals a double physis was formed in the metaphyseal area. This was obviously due to the separation of proliferative chondrocytes in the metaphysis as a result of distraction. After consolidation of the distraction area, this zone of chondrocytes, located in the metaphysis, was still producing new bone 20 weeks postoperatively.

It has been shown experimentally that after gradual physeal distraction a new metaphysis is formed (Ring 1958, Ilizarov and Soybelman 1969, Monticelli and Spinelli 1981, Peltonen et al. 1984a). According to Monticelli et al. (1981) and de Pablos et al. (1986), separation of the physis occurs through the layer of hypertrophic chondrocytes. After resorption of the hematoma from the distraction area, collagen bundles can be seen organized in the direction of the distraction (Monticelli et al. 1981). The gap is then gradually filled with trabecular bone, which is formed from the inner layer of the periosteum and from the epiphyseal and metaphyseal sides (Ring 1958, Peltonen et al. 1984a). According to De Bastiani et al. (1986), a quite different mechanism of bone formation occurs when the physeal distraction is carried out slowly, which is called chondrodiatasis, without causing a fracture between the epiphysis and metaphysis; bone lengthening is achieved by hypertrophy of the physis and acceleration of the enchondral osteogenesis.

The exact mechanism of the metaphyseal healing is, however, not well known. Also the quality of the bone

formed in the distracted area has not been clarified. We studied the mode of bone formation after physeal distraction and the effects of the distraction on the physis itself.

Animals and methods

Using an external fixation device (Peltonen et al. 1984a), gradual physeal distraction of the distal radius was performed on 18 still-growing, 2-5-month-old sheep. Kirschner pins were drilled percutaneously, a double external frame was applied, and distraction was started at a rate of 0.5-1.0 mm/day. Radiographs were taken regularly during the distraction and from cut bone specimens. The distraction was continued for 2-4 weeks, and the external fixation was removed when the operated on radius was clinically and radiographically consolidated. The animals were killed after 1, 4, 5, 6, and 7 weeks, and 2, 3, 4, and 5 months. Before death, 50 mg/kg of oxytetracycline was given intramuscularly.

The operated and nonoperated on radii were sawed in the coronal plane, and the specimens were photographed in reflected fluorescent light and then fixed in buffered formaldehyde (pH 7.2) for histologic examination. The decalcified bone specimens were stained in hematoxylin and eosin, van Gieson, and Alcian-blue. The histologic preparations were also examined under polarized light microscopy.

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Figure 1A



Figure 1B



Figure 2

Figure 1. Physeal distraction of the radius in sheep.
A. Six weeks postoperatively. Bone is formed from the metaphyseal and epiphyseal sides.
B. Consolidated distraction area (arrow), 4 months postoperatively.

Figure 2. Oxytetracycline incorporation in the distraction area composed of bone trabeculae. The physis is still open 4 months postoperatively.

Results

After 5-7 days of distraction, separation of the physis from the metaphysis occurred through the hypertrophic layer of chondrocytes. Just after the lysis occurred, the gap was filled with blood. The first radiographic signs of ossification were observed 4 weeks later. In tetracycline studies, it was seen that the bone forma-

tion occurred from the inner layer of the periosteum, from the metaphysis, and from the epiphyseal side towards the center of the distraction area. Consolidation occurred on an average within 8 (5-12) weeks postoperatively (Figures 1 and 2). The length gained was 4-16 mm and averaged 7 percent of the control radius at the termination of the experiment.



Figure 3

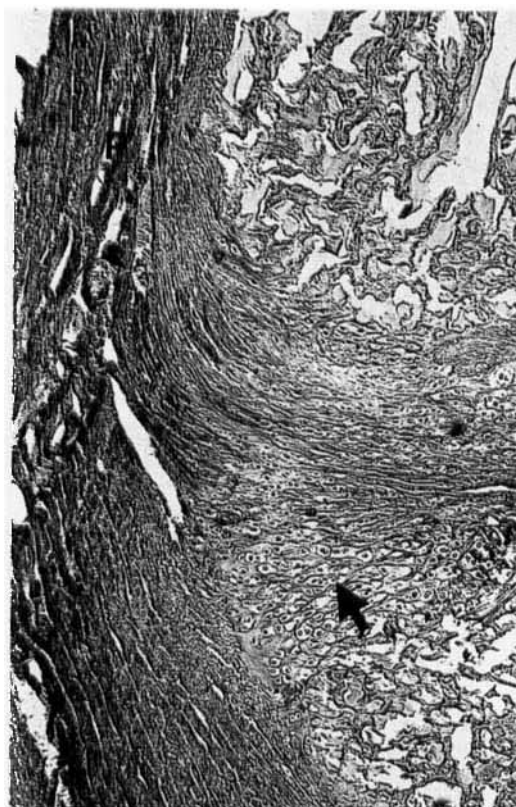


Figure 5

Prior to bone formation, collagen bundles were first organized in the direction of the distraction. Bone formation occurred from the epiphyseal and metaphyseal sides. Under polarized light microscopy, lamellar trabecular bone was seen formed along the collagen bundles (Figure 3). Osteoblasts accumulated at the junction of the bone and the collagen matrix. In the periphery of the distraction area, woven bone was formed from the inner layer of the periosteum (Figure 4). Also in some places where the collagen was disorganized, woven bone was observed. From the perichondrium,



Figure 4

transverse inclusions, surrounded by chondrocytes and woven bone, were penetrating into the metaphyseal distraction area (Figure 5). These inclusions were shifted more proximally in later stages of the follow-up.

In the metaphyseal area, also separate groups of chondrocytes were seen in some specimens; it seemed that enchondral bone formation occurred in these areas. At both ends and in the periphery of the distraction area, capillary proliferation and dilatation were observed.

From 8 weeks postoperatively, the center of the distraction area was mainly composed of lamellar trabecular bone. Areas of woven bone near the periosteum and in some places in the metaphyseal area were gradually remodeled into lamellar bone. Osteoclasts could be seen in the boundary zone between the woven

Figure 5. A transverse inclusion is penetrating from the perichondrium (P) into the metaphyseal distraction area 7 weeks postoperatively. Near the penetrating bud, chondrocytes (arrow) can also be seen. Alcian-blue staining, x 14.

Figure 4. Woven bone is seen in the periphery of the distraction area 7 weeks postoperatively. The periosteum is marked with an arrow. Polarized light microscopy, x 33.

Figure 3. Lamellar trabecular bone is formed as evidenced by the collagen bundles in the direction of the distraction 6 weeks postoperatively. Polarized light microscopy, x 33.



Figure 6

Figure 6. Tetracycline incorporation (arrows) on both sides of the zone. Double physis, located in the metaphysis of the distracted radius 20 weeks postoperatively. e = epiphysis.

Figure 7. A. A zone composed of chondrocytes through the metaphysis of the operated on radius (x 33). B. The distal half of the zone, which resembles the configuration of the physis, with the chondrocytes organized in palisades (Alcian-blue staining, x 66).



Figure 7A

and lamellar bone, indicating an active remodeling process.

From 16 weeks on, the distracted area was, for the most part, filled with lamellar bone trabeculae. In two sheep, 18-20 weeks after the operation (9-11 weeks after removal of the fixation), a narrow zone through the metaphyseal area was composed of chondrocytes; cells resembling proliferative chondrocytes were observed in the center of the zone. At both ends, the cells became hypertrophic, and active osteogenesis occurred in both directions in the zone. Histologically, the configuration of this zone resembled that of the physis. Tetracycline was incorporated in both sides of the zone, indicating active bone formation (Figures 6 and 7).

The epiphysis of the operated on bone became 2 (0-4) mm wider than that of the control bone specimen. In some instances, focal necrosis and inclusions from the physis penetrated the metaphysis. These inclusions were usually located near the pin tract in the bony epiphysis. The physis around the inclusions and sporadic necrotic areas was apparently undisturbed.

In all the sheep but 1, which were followed up to 20 weeks, the physis was open after the distraction. In 1 animal the physis was prematurely closed 15 weeks postoperatively. In another sheep the ossification of the distraction area was disturbed: viz., areas of woven bone were noted in the connective tissue 3 months after the operation, and the physis was also deformed and in some places closed. In most of the animals, however, the configuration of the physis corresponded to that of the control radius.

Discussion

It is obvious that collagen is essential for the bone formation during the distraction (Peltonen et al. 1987). The collagen bundles organized in the direction of the distraction apparently form a template for bone formation (Monticelli et al. 1981, Connolly et al. 1986). In our study, it was observed that if the collagen bundles were organized lineally; lamellar trabecular bone was formed from both ends of the distraction gap. In the periphery, near the periosteum, and in areas where the collagen was disorganized, woven bone was formed as in ordinary callus tissue (McKibbin 1978). According to Ring (1958) the consolidation occurs first in the periosteal area, where a cylinder of bone is formed to encircle the lengthened segment. Also in our study, active formation of woven bone from the inner layer of the periosteum was observed.

We therefore suggest that in distractional bone healing, two pathways of bone formation occur. It is proba-

ble that distraction is the main factor causing the organization of collagen bundles, which is required for lamellar bone formation. If this organization is disturbed, woven bone is first formed, which is then remodeled into lamellar bone, as in the periosteal area.

In some cases, groups of chondrocytes were found in the metaphyseal area after distraction; in 2 animals a zone of chondrocytes through the metaphysis was formed. It was obvious that even after 20 weeks this zone, functioning as a double physis, was still producing bone in two directions by enchondral osteogenesis. It is possible that in these cases a layer of proliferative chondrocytes had been separated at the start of the distraction and migrated into the metaphyseal area, where the bone formation continued as in physal bone growth. Connolly et al. (1986) reported that epiphyseal distraction occasionally causes the separation to occur through the layer of proliferating chondrocytes of the physis. The other source of the chondrocytes wandering into the metaphysis could be the perichondrial area. We observed that during the distraction, transversal inclusions from the perichondrium, containing also chondrocytes, tended to penetrate into the metaphyseal side of the distraction area. It is possible that the physal separation is not so uniform in the perichondrial area due to the dense fibrous structure of the perichondrium. Also the organization of collagen in different directions in the perichondrial ring (Brighton 1978) could affect the separation.

Some controversy exists concerning the effects of the distraction on the physis. Jani (1975) and Fishbane and Riley (1978) reported premature physal closure of the operated on limb. Changes in the organization of the chondrocytes of the physis were noted by Monticelli et al. (1981). It has also been suggested that the harmful effects of the distraction are dependent on the rate of distraction and the force used to cause the physal separation (De Bastiani et al. 1986, de Pablos et al. 1986). Peltonen et al. (1984b) observed that separation of the physis occurred more easily in younger animals and that the healing was also rapid. Complications were also more common in animals closer to skeletal maturity. We observed local changes in the physis after distraction; but in spite of this, physal growth continued in most of the animals after consolidation of the distraction area.

It is known that the diameter of the physis increases with longitudinal growth of the bone by probably appositional growth in the perichondrial area (Tonna and Cronkite 1963, Solomon 1966). The mechanism of induction of the latitudinal epiphyseal growth is, however, not known. The increase of the epiphyseal diameter by distraction, observed by us, could be due to the increased vascularity in the perichondrial area.

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