Bone formation after distraction osteotomy of the radius in sheep

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Gradual distraction by external fixation was performed one week after osteotomy of the radius in 12 sheep. Bone regeneration in the distraction area was studied by light and electron microscopy. One week after starting the distraction the gap was composed of hematopoietic cells and fibroblasts. The collagen production had already started and it continued actively throughout the distraction period. The collagen produced by the fibroblasts in the central interzone of the gap was organized according to the direction of the distraction. The osteoblasts were lined up along the collagen bundles and osteoid formation was most active around the capillaries. Mineralization started two weeks after the beginning of the distraction. After cessation of the distraction, separate groups of cartilaginous cells were found in some specimens among the newly formed bone. Our findings suggest that osteogenesis as a result of gradual distraction occurs through the whole distraction area with preceding formation of organized collagen matrix. The bone structure in the distracted segment represents an organized lamellar structure at an early stage of the osteogenesis.

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The basic biological phenomena occurring during distraction of tissues have been described by Ilizarov (reviewed 1989 a,b). In distraction osteogenesis it has been suggested that bone forms by membranous ossification (Tajana et al. 1989, Aronson et al. 1990).

In physeal distraction, new bone is formed from the intact periosteum as well as from the epiphyseal and metaphyseal sides of the distraction area (Ring 1958, Monticelli and Spinelli 1981, Steen et al. 1987, Peltonen 1988). At the metaphyseal or diaphyseal levels, when the distraction is carried out after an open osteotomy or corticotomy, bone is formed centripetally from the osteotomized bone ends towards the center of the gap (Alho et al. 1982, Peltonen et al. 1988, 1989, Aronson et al. 1990). Recent studies have revealed that formation and organization of collagen matrix into the distraction area precedes the bone formation (Vauhkonen et al. 1990). According to Monticelli et al. (1981) and Connolly et al. (1986) the distraction gap in physeal distraction is first filled with hematoma, which is gradually replaced by fibrous and collagen tissue. As a result of distraction the forming bone is also organized in the direction of the distractive tensile force. The same kind of bone regeneration has been shown to occur in cortical bone distraction (Delloye et al. 1990).

During the distraction, the central interzone of the distraction area remains radiotransparent as long as the distraction proceeds (Alho et al. 1982, Aronson et al. 1990) and is closed by bone after cessation of the distraction. According to Ilizarov (1989a), this interzone is composed of longitudinally oriented osteoid trabeculae. In the study by Kojimoto et al. (1988), the central area was composed of longitudinal fibers lined with proliferating cells.

We analyzed the early cellular changes during the distraction and the formation and quality of the new bone in the lengthened segment.

Material and methods

An external fixator was applied to the radius of 12 sheep. The animals were anesthetized for the operation by giving 0.3–0.5 mL of 2 percent xylatsin solution (Rompun®) intravenously. The bone was osteotomized at the distal metaphyseal-diaphyseal level by chisel, in order to preserve as much as possible of the medullary and periosteal structures. Gradual distraction was started after one week of neutral fixation as described in a previous study (Peltonen et al. 1988). The rate of

Table 1. Postoperative follow-up time (weeks) and grouping of the animals

n	Distraction time	Follow-up time
2	3 and 4	4
1	4	5
1	5	6
1	3	7
2	3 and 4	11
1	4	14
2	4 and 6	16
1	6	17
1	4	28

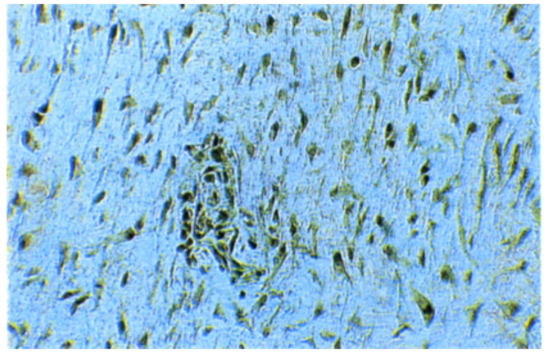


Figure 1. A biopsy specimen from the distraction area after 1 week of distraction (2 weeks after the osteotomy). Hematopoietic cells and fibroblasts are seen. Beginning of capillary formation in the distraction area. Van Gieson staining, ×190.

distraction was 0.5 mm/day and it was continued for 3–6 weeks (Table 1). The fixation device was removed when the operated bone was considered clinically and radiographically stable.

Sequential biopsies were taken from the distraction area in four sheep once weekly during the period of 1–4 weeks after starting the distraction (2–5 weeks after the osteotomy). The core biopsies were taken through the center of the distraction area, which was determined radiographically, by a needle of 2 mm in diameter, perpendicularly to the long axis of the bone. The animals were killed 4–28 weeks postoperatively. Both radii were sawn in the frontal plane and one half of the distracted segment was further processed for histological examination. The non-operated contralateral radius was used as a control. The specimens for light microscopic studies were fixed in buffered formaldehyde (pH 7.2) for 2–3 weeks, whereafter they were decalcified in 30% formic acid, embedded in paraffin and cut into sections of 4–6 μm. Hematoxylin and eosin, van Gieson and alcian blue stainings were performed. Polarized light microscopy was used to examine the bone structure.

Samples for transmission electron microscopy (TEM) were taken from the biopsies and from the animals killed four weeks after starting the distraction. The specimens were 2 × 2 mm in size. They were taken from the center of the gap and from the junction of the new bone and connective tissue in the distraction area. The specimens were immediately fixed in 3% glutaraldehyde buffered with 0.1 M phosphate buffer (pH 7.2) for 2 hours at 20 °C. After rinsing in several changes of phosphate buffer, the samples were postfixed in phosphate-buffered 1% osmium tetroxide for one hour at 4 °C. After dehydration in acetone, the samples were embedded in Epon. For light microscopy prior to TEM, 1–2 μm sections were cut and stained with 1% solution of toluidine blue in saturated sodium bicarbonate. For TEM, thin sections were double-stained with lead citrate and uranyl acetate at 20 °C, and examined in a JEOL JEM-100S transmission electron microscope.

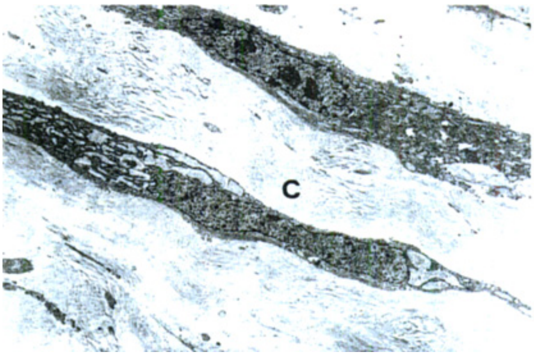
Results

The distraction gap was about 4 mm wide one week after starting the distraction (2 weeks after the osteotomy). The biopsies taken at this stage showed that the cell population in the distraction area was heterogeneous. Red blood cells and other hematopoietic cells with some fibroblasts and fibroblast-like cells were seen with light and electron microscopy. A beginning of capillary formation into the distraction gap was seen (Figure 1). The collagen bundles were thin and not organized. A few osteoblasts were scattered at the surface of the collagen fibers.

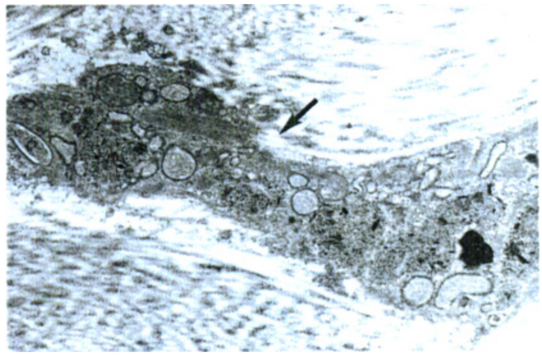
At 2 weeks of distraction, the center of the distraction gap was mainly composed of longitudinally-oriented fibroblasts, which were producing collagen fibers (Figure 2). The collagen bundles were coarser than at the first week's specimens. The capillaries were thicker and more numerous than a week earlier. Osteoblasts were seen along the surface of the collagen bundles and new bone trabeculae. A beginning of the mineralization was evident at this stage of distraction (Figure 3).

The specimens from the third week revealed more advanced mineralization of the distraction area. Osteoblasts were organized along the collagen bundles, as were the capillaries. The osteoblasts formed osteoid which was then mineralized. The mineralization proceeded toward the center of the distraction gap. The new bone trabeculae were oriented according to the collagen frame and displayed mostly a woven struc-

Figure 2. Electron micrograph (EM) from the distraction area after 2 weeks of distraction (biopsy specimen).



The fibroblasts are oriented longitudinally as also are the collagen bundles (C), ×1150.



EM of a fibroblast-like cell which obviously secretes the collagen fibrils (arrow) into the distraction area, ×8750.

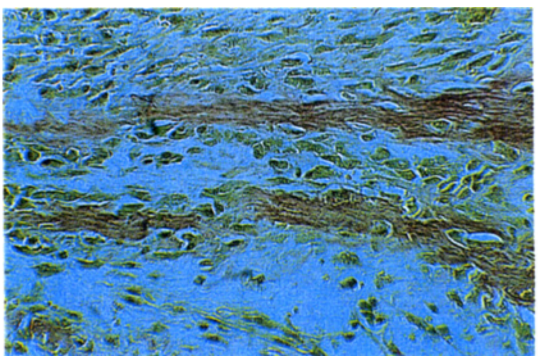


Figure 3. A biopsy specimen after 2 weeks of distraction. The osteoblasts are lining the collagen bundles and newly-formed bone trabeculae. v G, ×230

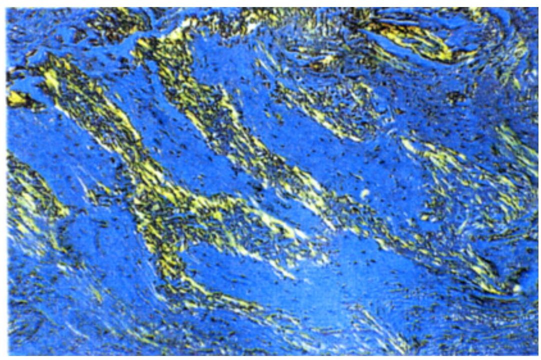


Figure 4. The specimen at 3 weeks of distraction shows the early bone formation. The structure of the bone trabeculae resembles woven bone. Polarized light microscopy, ×80.

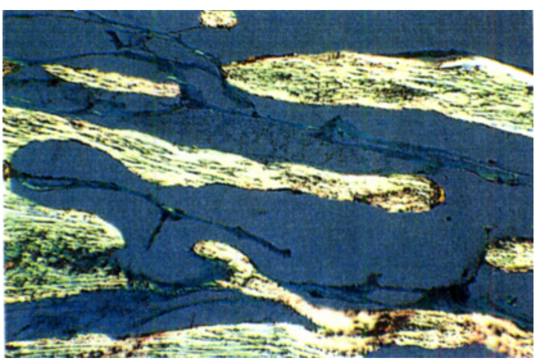


Figure 5. Bone trabeculae at 4 weeks of distraction. Lamellar organization of the bone structure is evident. Polarized light microscopy, ×60.

ture (Figure 4). In some places also chondroblasts were seen as isolated groups among the bone and collagen areas. In TEM some myelinated and unmyelinated nerve fibers were noted in 3-week specimens.

4 weeks after distraction was started, the center of the distraction gap was mainly composed of fibroblasts and organized collagen. The center of the gap did not differ from that of the 3-week specimens. The osteoid formation and mineralization occurred from the osteotomized bone ends centripetally. In some places separate groups of cartilaginous cells were seen. At the periphery of the distraction gap, bone formation occurred, apparently derived from the periosteum. The structure of the new bone was more lamellar and oriented according to the direction of distraction (Figure 5).

The distraction area was consolidated after cessation of the distraction, within 3–4 weeks. Histologically, the consolidated bone segment was composed of a network of trabecular bone, which was mainly organized in the direction of the distractive force. In some places separate chondroid islands were seen among the newly formed bone.

In the specimens taken 16–28 weeks postoperatively, the lengthened segment was fully consolidated, except in two cases. However, formation of the new cortex and remodeling of the bone structure was not completed. The marrow cavity was for the most part open and the lengthened bone area was composed of lamellar bone. In one case the bone formation was disturbed and pseudoarthrosis developed. Chondroid and disorganized fibrous tissue predominated in the distracted segment. In another animal the bone formation was not completed and an angular deformity of the lengthened segment had developed.

Discussion

The early cell population and capillary proliferation in the distraction gap are essentially the same as seen in fracture healing (Frost 1980). In fracture repair the first stage is followed by a fibrocartilaginous phase, which precedes the bony phase (Urist et al. 1965). Controversial opinions have been presented concerning the osteogenesis during distraction. In the studies by Tajana et al. (1989) and Aronson et al. (1990) formation of primary cartilaginous callus as seen in endochondral ossification was not observed. However, in the study by Kojimoto et al. (1988), it was suggested that bone formation during gradual distraction occurs via the cartilaginous phase. In the study by Alho et al. (1982), both periosteal and endosteal callus formation participated in the bony healing of the distraction area. In their study both chondrogenesis and osteogenesis were observed.

In our study sequential biopsies were taken from the distraction area. Although the traumatic effect of the biopsies taken weekly cannot be neglected, morphological findings that could have been interpreted as consequences of the biopsies were not found. We found that the next stage after the initial phase was formation of the interzone composed of collagen-producing fibroblasts and longitudinally-oriented collagen bundles between the osteotomized bone ends.

Collagen production continued in the central part and at the same time mineralization and bone formation progressed from the osteotomized bone ends towards the center of the gap. Biopsies from human tibiae after 20–30 days of distraction lengthening have also revealed the formation of organized collagen and capillary network in the distraction area (Tajana et al. 1989). Several previous studies have also shown that formation of an organized collagen frame before bony consolidation occurs in metaphyseal, diaphyseal and physeal distraction (Monticelli et al. 1981, Connolly et al. 1986, Ilizarov 1989a, Peltonen et al. 1988, 1989). In the study by Vauhkonen et al. (1990), it was shown that the collagen matrix, composed of type I collagen, was rapidly formed during the first weeks of distraction. It is likely that continuous "de novo" collagen synthesis by the fibroblasts occurs at the immature central part of the gap, as long as distraction is continued.

Mineralization and bone formation occur centripetally from the osteotomized bone ends toward the center of the gap (Alho et al. 1982, Peltonen et al. 1988, Aronson et al. 1990). In our study it was found that the osteoblasts were arranged, often as a monolayer line along the collagen bundles and around the newly-formed capillaries. The mineralization started after two weeks of distraction, as also found by Ilizarov (1989a) and Delloye et al (1990). In the study by Delloye et al. (1990), medullary and periosteal osteogenesis were particularly active at 2 weeks. The organization of the collagen bundles according to the distractive tensile force explains the orientation of the new bone. Although lamellar organization of the bone was evident after 4 weeks of distraction, it was preceded by a woven bone phase. According to the study of Shapiro (1988) cortical bone defect is healed initially by woven bone, followed by a rapid lamellar replacement at 4 weeks. In his study a drill hole of 2.4 mm in diameter was made in the diaphysis of the femur and tibia of rabbits.

The bone lamellae were arranged mostly parallel at 4 weeks of distraction. After cessation of the distraction and consolidation of the lengthened segment the bone structure had developed into a trabecular network filling the distraction area. The same bone structure was described by Tajana et al. (1989). Controversial opinions exist concerning the final cortical remodeling after bone lengthening. In physeal distraction (Monticelli et al. 1981) complete reconstruction of the lengthened bone segment occurred after 12 months. According to Ilizarov (1989a), cortical bone formation occurs much earlier, even after 3–4 weeks of bone distraction. In our study, complete corticalization of the distracted segment was not observed within the follow-up period up to 28 weeks.

Some aspects seem obvious in distraction osteogenesis: 1) during the distraction a collagen synthesizing interzone and new capillaries are formed in the distraction area; 2) the interzone is closed by bone after cessation of the distraction, provided that bone formation during the distraction has been sufficient; and 3) osteogenesis occurs centripetally and the bone displays early lamellar organization according to the collagen template.

Whether the fibrocartilaginous phase, as seen in enchondral bone formation, is passed in distraction osteogenesis, is not quite clear. In the study by

Delloye et al. (1990) enchondral ossification around the elongated zone, and separate areas of cartilage in the distracted segment were noted. In our study, cartilage tissue and groups of cartilaginous cells were evident in the bone. Ilizarov (1989a, b) has suggested that in optimal conditions, e.g., stable fixation and fractionated distraction rate, not exceeding 1 mm/day, the bone forms during the distraction straight to the organized matrix without a cartilaginous phase. According to Paley (Paley 1988, Paley et al. 1990) the stability of the frame and rate of distraction affect the mode of osteogenesis during distraction. The case in our study with development of non-union and cartilaginous and fibrous tissue accumulation was probably due to insufficient stability of the fixation.

The biologic mechanism explaining how gradual distraction affects the osteogenesis is still unclear. Studies on the mechanical factors influencing the local mediators in bone repair are needed to answer the unresolved questions.

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