BONE LENGTHENING IN RABBITS BY CALLUS DISTRACTION

THE ROLE OF PERIOSTEUM AND ENDOOSTEUM

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The histology and mechanics of leg lengthening by callus distraction were studied in 27 growing rabbits. Tibial diaphyses were subjected to subperiosteal osteotomy, held in a neutral position for 10 days and then slowly distracted at 0.25 mm/12 hours, using a dynamic external fixator. Radiographs showed that the gap became filled with callus having three distinct zones. Elongation appeared to occur in a central radiolucent zone; this was bounded by two sclerotic zones.

Histologically, the radiolucent zone consisted of longitudinally arranged cartilage and fibrous tissue while the sclerotic zones were formed by fine cancellous bone. New bone occasionally contained islands of cartilage, suggesting it had been formed by endochondral ossification. After completion of distraction, the two sclerotic zones fused, shrank and were eventually absorbed, leaving tubular bone with a new cortex.

When the periosteum had been removed at the operation, callus formation was markedly disturbed and there was failure of bone lengthening. Scraping of endosteum, in contrast, did not have a pronounced effect. These results suggest that the preservation of periosteum is essential if bone lengthening by callus distraction is to succeed, and that preservation of the periosteum is more important than careful corticotomy.

Callus distraction (callotasis) is one method of lengthening a long bone without grafting. After a diaphyseal osteotomy, the early callus is elongated by slow, progressive axial distraction using a dynamic external fixator. Recent advances in surgical techniques and equipment have made bone lengthening by callotasis both easier and safer, and many successful clinical cases have been reported (De Bastiani et al. 1987; Wakisaka et al. 1988). However, only limited information is available regarding the basic mechanisms involved in callus elongation. Various questions arise; does the callus fracture or does it elongate intact? Is the whole callus elongated or is there a specific site of cell proliferation? Is the mode of ossification endochondral or intramembranous?

Our study was designed to demonstrate the histomechanics of callotasis and to determine the role of periosteum and endosteum in callus formation.

MATERIALS AND METHODS

The experimental study was carried out on 27 immature Japanese white rabbits weighing about 2.0 kg. A unilateral dynamic external fixation device (Orthofix M-100, Fig. 1) was applied to the right tibia which, in the rabbit, is fused with the fibula in its distal half. The

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operation was performed under nembutal and fluothane anaesthesia under aseptic conditions with prophylactic antibiotic cover by intravenous cefotiam dihydrochloride. A longitudinal skin incision was made on the medial aspect of the tibia. The periosteum was incised longitudinally and carefully retracted. Four self-tapping tapered screws were placed at right angles to the longitudinal axis of the shaft using a special screw guide. Transverse osteotomy was performed just below the tibio-fibular junction between the second and third screws, using a hand saw. The screws were then clamped to the external fixation device, so that the proximal and distal bone fragments could be repositioned. A number of animals were then allocated to each of the three experimental groups.

Group 1 – (14 animals). The periosteum was sutured and the bone ends were repositioned.

Group 2 – (8 animals). Periosteum was removed from the segments between the second and third screws before the bone ends were reduced.
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RESULTS

Radiographs

Preliminary experiment. Five animals in Group 1 were used in the preliminary experiment to determine the timing of distraction after operation. Bone lengthening was achieved successfully after a seven to 10 day waiting period. Distraction immediately after operation resulted in retardation of callus formation, while excessive delay allowed premature bony fusion of the tibial osteotomy. The waiting period was set at 10 days for all subsequent experiments.

Group 1. Bone lengthening was successful in this group, with a maximum elongation of 2.4 cm and a maximum follow-up of six months. Figures 2 to 9 show the radiographic changes during this experiment. During the waiting period, the osteotomy site became surrounded by immature cloudy callus (Fig. 2). As distraction began, the bridging callus separated into proximal and distal segments (Fig. 3). It was considered likely that elongation was occurring at the central radiolucent zone which was bounded by proximal and distal sclerotic zones (Fig. 4). By four weeks, new zones with low radiodensity appeared outside the sclerotic zones (Fig. 5). The central radiolucent and the adjacent sclerotic zones were maintained at a relatively constant thickness during the distraction period (Fig. 6). After the completion of distraction, the two sclerotic zones became fused (Fig. 7), then shrank (Fig. 8) and were eventually absorbed (Fig. 9). At the end of the experiment there was tubular bone with a new cortex.

Group 2. When the periosteum had been removed at the operation, callus formation was seriously compromised and the experiment was often interrupted by fracture. Figures 10 and 11 show examples of the radiographic appearance in this group, and show that only a small amount of endosteal callus was produced.

Group 3. When the bone marrow cavity had been scraped and the periosteum preserved, no obvious disturbance of callus formation was detected radiographically. The quantity of callus was slightly decreased in the initial stages of distraction (Fig. 12), but increased in the later stages (Fig. 13). The characteristic zonal structure seen in Group 1 was again observed.

Histology

The histological findings in each group corresponded well with the radiographic appearances.

Group 1. Figures 14 and 15 are longitudinal sections of a tibia lengthened 4.0 mm and stained with Azan-Mallory and toluidine blue, respectively. The radiographically

Group 3 - (5 animals). The endostem was scraped out from both bone ends between the second and third screws before the periosteum was sutured and the bone ends were repositioned.

Five animals in Group 1 were used for a preliminary experiment to define the conditions of distraction. In the subsequent experiments, distraction started routinely 10 days after operation at a rate of 0.25 mm every 12 hours (0.5 mm/day). The process of new bone formation was monitored by weekly radiographs and at various stages of elongation animals were killed for histological examination of the bone. Decalcified 3 μm sections were stained for light microscopy with haematoxylin-eosin, toluidine blue and Azan-Mallory stains.
sclerotic callus consisted of fine cancellous bone. External callus originating from the detached periosteum expanded around the proximal and distal diaphysis whereas endosteal callus was limited to the mouth of the bone marrow cavity. The area of the central radiolucent gap was filled with elongated cartilage, areas of fibrous tissue and haemorrhage. Cartilage in the circumferential region was elongated without a break. Some longitudinal fibres were seen to bridge the whole length of the distraction gap without interruption. The proximal and distal ends of the longitudinal fibres merged with fibrocartilage which contained hypertrophic chondrocytes arranged in columnar fashion (Fig. 16). New bone was formed in the adjacent sclerotic zone.

Figure 17 shows the distribution of cartilage in a transverse section of the same specimen as in Figure 15. The distraction gap is surrounded by a complete circle of metachromatic cartilage. The origin of this cartilage was presumed to be the peeled periosteum, because the cartilage collar was formed only when the periosteum had been repaired at the time of operation. By the same token, the cartilage islands found in the central region may be derived from the endosteum.

Figure 18 is a longitudinal section of a tibia lengthened 15.0 mm. The histology is seen to reflect the characteristic zonal structure shown radiographically in Figure 6. The central radiolucent zone is composed predominantly of longitudinal fibres lined with prolifer-
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Fig. 18

Figure 18 - Longitudinal section of a tibia lengthened 15.0 mm (Azan-Mallory X 10).

Fig. 19

Transitional region from cartilage to bone (toluidine blue).

Fig. 20

Cartilage remnants in new bone (toluidine blue X 200).

ating cells. The circumferential cartilage collar found in the early stages of distraction (Fig. 15) had been replaced by fibrous tissue at this stage. Most longitudinal fibres were intact despite elongation; they merged with fibrocartilage at their proximal and distal ends. The matrix of hypertrophic chondrocytes had been invaded by cells derived from bone-marrow; new bone had been formed on the surface of the eroded cartilage (Fig. 19). Fine cancellous bone in the adjacent sclerotic zone occasionally contained islands of cartilage remnants (Fig. 20). The centre of the sclerotic zone had been absorbed in a rate-limited manner from the bone-marrow side (Fig. 18). Bony trabeculae in the circumferential region had increased in thickness and linked with the periosteal callus to form a new cortex. The original cortex was eroded and thinned down.
**Group 2.** The removal of the periosteum caused a deficiency in the formation of external callus (Figs 21 and 22). Although endosteal callus was formed normally during the waiting period of 10 days, the amount of callus was insufficient to begin distraction.

**Group 3.** Scraping of the bone marrow cavity completely prevented endosteal callus formation. However, no obvious effects were observed on external callus formation (Figs 23 and 24). The osteotomy site was surrounded by a delta-shaped cartilaginous collar.

**DISCUSSION**

Our study has provided information regarding the basic mechanisms involved in bone lengthening by callus distraction. Very reproducible and homogeneous results were obtained because the experimental conditions were controlled by a rigid system of external fixation. Slow axial distraction of the developing callus produced a characteristic zonal structure resembling a growth plate. Tissue elongation occurred only in the central radiolucent zone; the other zones contained calcified bone matrix. A similar zonal structure can be seen on careful review of the radiographs published with previous experimental reports (Ilizarov and Berko 1976; Alho et al. 1982) and clinical studies (Kawamura et al. 1968; De Bastiani et al. 1987; Wakisaka et al. 1988).

De Bastiani et al. (1986) reported that slow distraction of the epiphyseal plate at rates of 0.25 mm per 12 hours produced hyperplasia of the growth cartilage without evidence of detachment. Using the same distraction rates, our study has shown that some cartilage...
and longitudinal fibres in the central radiolucent zone can be elongated intact, although some longitudinal fibres were interrupted and this was associated with considerable haemorrhage. The origin of the cells which lie alongside the longitudinal fibres is uncertain, but they appeared to retain the potential to proliferate and to differentiate into chondrocytes. The columnar arrangement of the chondrocytes in the transitional region from cartilage to bone indicated that the mode of ossification was endochondral. Some longitudinal fibres, however, seemed to translate directly into new bone, suggesting the participation of another mechanism.

Previous studies on bone lengthening after osteotomy have emphasised the importance of protecting the bone marrow during operation and have recommended a carefully limited corticotomy (Kawamura et al. 1968; Ilizarov et al. 1969; De Bastiani et al. 1987; Dal Monte and Donzelli 1987). Our results, however, have demonstrated that the endosteum and bone marrow are not indispensable for adequate callus formation and that the periosteum is of particular importance. Our recent clinical experience of leg lengthening by callotasis has supported this experimental conclusion. Sufficient callus can be generated after subperiosteal transverse osteotomy using an electric bone saw with continuous cooling of the osteotomy site (Wakisaka et al. 1988; Yasui, personal communication). We therefore consider that preservation of the periosteum is more important than careful corticotomy.

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REFERENCES


