HISTOLOGY OF A LENGTHENED HUMAN TIBIA

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We describe the histology of a specimen taken from an amputated leg seven months after a 15 cm bone gap in the tibia had been closed by bone transport.

Lengthening appeared to have occurred by repeated minor trauma to the bone, with the fractured trabeculae in sufficiently close contact for the repair process to proceed. Osteogenesis did not occur through a cartilage phase, but the fracture gaps were bridged by collagen fibres, around which new bone formed. Microfractures had repaired by primary healing with woven bone and with no microcallus. Small regions of bone were necrotic. Resorption of the necrotic bone and remodelling of the immature bundle and woven bone were still at an early stage, suggesting that complete remodelling in man may take years rather than months.

The technique of lengthening bones by stretching callus in its early stages of formation was first described in the last century and the subject has been recently reviewed by Moseley (1989) and Paterson (1990). However, little is known about the way in which new bone forms during distraction or about the characteristics of its matrix. Histological studies of the lengthened bones of dogs (Aronson et al 1989; Ilizarov 1989; Delloye et al 1990) and sheep (Monticelli, Spinelli and Bonucci 1981) have established that most new bone forms around collagen fibres that bridge the fracture gap, unlike osteogenesis in normal fracture repair when a cartilaginous callus is usually present. Cartilage or fibrocartilage only formed during distraction if the fixator allowed displacement between the bone ends (Ilizarov 1989).

The relevance of these animal studies to man is uncertain, and we believe that only one histological study has been carried out in man (Tajana, Morandi and Zembo 1989). Therefore, when a human tibia which had been lengthened by 15 cm had to be amputated, a unique opportunity arose to examine the structure of the newly lengthened bone matrix.

CLINICAL HISTORY AND METHODS

A 19-year-old man, involved in a motorcycle accident, suffered extensive soft-tissue injuries and lost 18 cm of bone from the distal half of one tibia. The neurovascular state of the limb was satisfactory, but there was extensive loss of the muscles of the anterior compartment. An external fixator was applied, with the limb immobilised 3 cm short of its normal length. Soft-tissue cover was achieved at four weeks by rotation flaps and skin grafting. The fixator was changed 14 weeks after injury for a 'Shearer' unilateral lengthener with a bone transport system (De Puy Thackray, Leeds, England). A corticotomy of the upper tibia was carried out and 11 days later, when some initial callus could be seen, bone transport was commenced at a rate of 1 mm a day in four increments. At various times the transport had to be stopped as radiographs showed failure of the callus to keep up with the distraction process. At other times the rate was increased to 1.5 mm a day. At the same time an attempt was made to lengthen the distal part of the tibia to regain the remaining 3 cm of overall limb length. The process was stopped after eight months when the transported section of the tibia had been moved 15 cm. It had not been possible to regain all the lost leg length and the limb remained 3 cm short. In a further operation the lower end of the transported bone was secured to the distal tibia with an interfragmentary compression screw and bone allograft was applied.

Four months after the lengthening ceased, union had occurred and the external fixator was removed and replaced by a below-knee patellar tendon bearing cast. One month later the patient was allowed to start partial weight-bearing. The cast was removed after a further two months and the patient encouraged to bear full weight. Soon afterwards he developed severe swelling of the foot and ankle distal to the lower fracture site and superficial ulceration on the dorsum of the foot. Admission to hospital with high elevation of the foot resulted in rapid healing but the patient was soon re-admitted with the same problem. After this had happened on two further occasions, the patient opted for a below-knee amputation, which was carried out seven months after...
lengthening had been stopped. The leg was amputated approximately 3 cm distal to the corticotomy site in the upper tibia. The amputated limb was immediately taken to the laboratory and the region of new bone was sawn longitudinally. Transverse slices were then cut at 1 cm intervals.

**Histology.** Small pieces of bone, taken at regular intervals from the lengthened region, were fixed in 10% neutral buffered formaldehyde, decalcified in several changes of 5% EDTA (in TRIS buffer, pH 7.4) over a period of two weeks, then processed through graded alcohols and chloroforms and embedded in paraffin wax. Transverse or longitudinal sections were cut at 8 to 10 μm; 50 to 80 sections were obtained from each block and a total of 50 blocks were cut. The sections were stained by the tetrachrome method of Ráisí and Ráisí (1975), which distinguishes new osteoid and immature unmineralised bone (blue) from mature fine-fibred calcified bone matrix (red).

**RESULTS**

**Gross appearance of the tibia.** Figure 1a demonstrates the extent of the bony defect at the onset of the bone transport procedure and the positioning of the unilateral lengthener. In Figure 1b, 12 months later, there has been distraction of 15 cm at the corticotomy site. The new bone is seen as linear calcification. Less radiodense bone is seen centrally and no cortex is apparent at this stage. The fracture ends, which were then in apposition, have been compressed and bone grafted. In Figure 1c the lengthener has been removed. Some angulation and collapse has occurred in the lengthened bone after weight-bearing. Overall alignment has been maintained and the distal fracture has united. Figure 1d shows the region of lengthened bone from the amputated tibia, sliced longitudinally.

**Histology of the lengthened bone.** Although a cortex was present throughout, it was much thinner (0.4 to 0.9 mm) than in a normal tibia. The cortical bone (Fig. 2a) stained unevenly, was very cellular and contained large spaces. Blood vessels were present in the spaces and active new bone formation could be identified by the presence of osteoid at many sites. Although some areas had begun to resemble compact cortical bone, the overall appearance was still that of coarse cancellous bone. The cancellous nature of the cortex was confirmed in transverse sections, where it could also be seen that the cortex was continuous with a relatively open network of bone trabeculae extending throughout the centre of the tibia. The spaces between the trabeculae contained loose connective and fibrovascular tissue. In some areas, particularly where the trabecular network was very open or absent, the formation of collagen fibres was extensive (Fig. 2b).
fibres were generally orientated longitudinally, parallel to the tensile force.

Bone matrix. By polarised light the bone matrix in the lengthened area was seen to contain much immature unmineralised bone (i.e., woven bone and bundle bone) (Fig. 3a). The origin of the bundle bone was at the interface between the bone and collagen fibres, where the incorporation of collagen fibres into new bone matrix could be seen (Fig. 3b). Immature bone was present in almost all trabeculae of the lengthened area, although the proportion of mature to immature bone varied. The continuity of fibre bundles with the bone trabeculae was also observed in longitudinal sections (Fig. 3c), where the fibres either passed into or emerged from the bone matrix.

Evidence of distraction. At the microscopic level, the effects of progressive distraction were evident as 1) wide gaps between trabeculae, 2) fissures in the bone matrix.
and 3) necrotic bone debris. Where the presence of wide gaps between bone trabeculae suggested that a displacement had occurred, fibrous material with the staining characteristics of collagen filled the gaps. Fissures in the bone matrix were frequently observed. If immature bone was still present at the centre of a trabecula, the fissures or 'microfractures' opened in this region, it being the weakest part of the trabecula (Fig. 4a). However, microfractures of the mature bone matrix were also seen. Some of these fissures might have been widened during sectioning, but it is unlikely that they were completely artefactual since many of the gaps were filled with immature bone. Figure 4b is a longitudinal section, showing open microfractures, and one microfracture filled in with fibrous material.

In many sections remnants of necrotic bone were observed. Necrotic bone could be identified by its ragged, shattered appearance and the absence of osteocytes in the lacunae. In some areas necrotic bone was adjacent to spicules of live bone (Fig. 5a). Fibrous material was frequently found around areas of necrotic bone. Small remnants of necrotic bone were found in almost every histological section. The very small pieces of necrotic bone that were observed in the spaces between trabeculae or within the central canal of an early haversian system (Fig. 5b) were probably the last remnants of dead, broken bone, left after most of the dead bone matrix had been resorbed.

**Bone remodelling.** Evidence of remodelling was found both on mature lamellar bone and on the still immature bundle or woven bone. Whenever capillaries were present in immature bone, concentric circles of lamellar bone were usually laid down on the immature bone. Furthermore, some trabeculae were undergoing resorption. However, the later stages of remodelling, such as the formation of secondary osteons or medullary remodelling, were not present.

Frequently, bone trabeculae of a fine cancellous structure were found around or between the struts of coarse cancellous bone as shown in Figure 6. The structure of these fine trabeculae suggests that the gap was originally filled with bundle bone, part of which had been replaced by lamellar bone around the vascular canals.

**DISCUSSION**

There has been only one previous histological study of a lengthened human tibia (Tajana et al 1989) and that was based on biopsies taken at various stages of distraction so that only small areas were sampled. In the present study, a large region of the lengthened tibia could be examined.

The histological appearances suggest that repeated distraction caused minor trauma of the trabeculae, as a result of which a bone repair response was initiated. However, there was continual interference from the progressive distraction forces and the whole region between the pins was subjected to tension stress each time a distraction was made. New bone was not only laid down at the cut ends but also in microfractures or gaps that appeared in the already lengthened bone.

The effects of the minor trauma were apparent as microfractures, wider gaps between bone spicules or as necrotic bone debris. The presence of microfractures seven months after cessation of lengthening may be because the lengthened bone remained plastic for some time after the lengthening process had been completed, as evidenced by the angulation which occurred at that site (Fig. 1c). However, the absence of cartilaginous callus implies that no excessive lateral movement took place (Ilizarov 1989). The presence of necrotic bone suggests ischaemia, possibly due to failure of the capillary network to keep up with the distraction process, although there had been no evidence of arterial insufficiency in the distal part of the limb.

**Distraction osteogenesis.** Previous studies have shown that in sheep the first event following the initial operation is the development of a haematoma (Monticelli et al 1981) while in man a clot containing colloids in a proteoglycan gel is formed (Tajana et al 1989). This stage could not be observed in the present study, but examples of subsequent stages could be seen. As described by Aronson et al (1989) and Ilizarov (1989) in dogs, new bone formed by two routes, neither of which involved an intermediate cartilage phase. Distraction osteogenesis therefore differs from endochondral bone formation at the growth plate and from osteogenesis during fracture repair.

The first route to new bone was found where the gaps between bone spicules were relatively wide. These gaps were bridged by collagen fibres like the fibrous bridging between bone fragments in delayed union or nonunion (Sevitt 1981b). Previous work had also identified collagen fibres as replacing the haematoma (Monticelli et al 1981) or the colloidal clot (Tajana et al 1989) of the initial fracture gap. Aronson et al (1989) and Ilizarov (1989) observed that the fibres fused with the newly formed bone trabeculae or were directly transformed into bone matrix. This fusion was also observed in the present study. In addition, several examples were seen of fibres acting as a substrate around which new bone matrix was deposited. The fibres themselves became incorporated into the bone giving rise to bundle bone.

The second route to new bone was seen following microfractures. If a trabecula contained immature bone, it was invariably in this region that the microfracture occurred, because it was the weakest point in the structure. However, cracks in fine-fibred bone matrix were also observed. In both cases the fissures healed directly with woven bone matrix, like the primary union of stable fissure fractures (Sevitt 1981a).

Whatever the route of initial bone formation, the end effect was that a fissure healed or a gap between two
Figure 4a – This transverse section shows a microfracture in a region of bundle bone (single arrow) and a crack in lamellar bone (double arrows) (× 230). b) several microfractures are seen in this longitudinal section (arrows), one of which (*) has been filled with fibrous collagen (× 230).

Figure 5a – Transverse section to show necrotic bone (*) adjacent to live bone. Part of the necrotic bone has become surrounded by fibrous collagen (× 230). Figure 5b – remnants of necrotic bone (*) are still present within the spaces between trabeculae and between haversian canals (× 230, see also Fig. 2a).

The older bone trabeculae (outlined) have been joined by newer trabeculae with a smaller mesh network (× 230).
bone spicules was bridged with new bone. A late stage of this process was seen when newer trabeculae were present adjacent to or between older bone spicules. This fine cancellous trabecular structure was similar to the first new bone trabeculae that are formed in a fracture callus. The new trabeculae thus extended the bone matrix into available space and even relatively distant bone spicules could be joined.

**Consolidation of immature bone.** The occurrence of bundle and woven bone has not been described in animal studies and may be a characteristic of human bone lengthening. The immature bone was not mineralised (Ralis and Ralis 1978) and must have been weaker than the mature fine-fibred bone. With time, the immature bone was replaced by fine-fibred bone, as ever-thickening lamellae around the inner perimeter of vascular canals. The prevalence of immature bone, the absence of secondary osteons and the relative absence of compact cortical bone indicate that remodelling was still at a very early stage, even seven months after the cessation of lengthening. The remodelling process may require years rather than months to produce mature, lamellar cortical bone.

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

**REFERENCES**


