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Assessment of Cell Proliferation in Regenerating Bone During Distraction Osteogenesis at Different Distraction Rates

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Summary: An experimental model of lengthening of the lower limb was used to study the morphology and cellular proliferation of regenerating bone tissue after 20% lengthening at four rates of distraction. Groups of rabbits were killed at different times 1-8 weeks after surgery. The regenerated area was divided into three zones: fibrous, primary mineralization front, and new bone. As the rate of distraction increased, the size of the fibrous zone increased and that of the new bone zone decreased. Necrosis, formation of cysts, and cartilage were found in the regenerated area at the higher distraction rates. Cell proliferation was assessed by *in vivo* labelling with bromodeoxyuridine, and the positive staining index for anti-bromodeoxyuridine antibody was calculated in the zones of the regenerated tissue. The index values for the fibrous zones and the new bone zones did not differ significantly in any of the groups. The value increased significantly ($p < 0.05$) in the primary mineralization front as the rate of distraction increased from 0.3 to 0.7 mm/day, but there was no further significant increase at higher distraction rates. In conclusion, cell proliferation was increased at all of the higher rates (more than 0.3 mm/day) of distraction studied. Higher rates of distraction caused tissue damage. A distraction rate of 0.7 mm/day appeared optimal for cell proliferation and histological characteristics.

The treatment of many congenital and acquired disorders of adult and developing skeletons necessitates the generation of new bone tissue in appropriate sites. Codivilla (6) first introduced the concept of limb-lengthening, whereby carefully osteotomized bone that is separated in a controlled manner generates new bone in the resultant gap. This phenomenon is termed distraction osteogenesis. The technique is now well established and has been applied diversely in orthopaedic reconstruction procedures (6,11,13). However, many aspects of distraction osteogenesis are still poorly understood.

The temporal sequences of events associated with distraction osteogenesis have been studied radiographically and histologically in animal models at distraction rates of 0.5 or 1 mm/day (2,7,14). Fast rates of distraction diminish the quality of the regenerating bone, and at slow rates there is a risk of premature union. Ilizarov (10) considered distraction at a rate of 1.0 mm/day most favourable for regeneration of bone. However, in clinical practice this rate often has to be decreased because of poor adaptation of the soft tissues or poor formation of bone due to the effects of metabolic states, drug usage, cigarette smoking, and systemic chronic diseases (19). The events following the osteotomy consist of an initial waiting period, a distraction period, and a consolidation phase. The distraction period continues as long as controlled gradual distraction is being applied (19). The consolidation period is the time following distraction when new bone is consolidated and remodelled. During the distraction period, histological examination shows that new trabeculae, which emanate from both ends of the bone that has undergone an osteotomy, terminate in a central fibrous interzone 4-8 mm wide (19). Detailed studies of the fibrous interzone (8,17) have demonstrated that spindle-shaped fibroblastic cells oriented parallel to the distraction force appear to gradually differentiate into osteoblasts and produce new bone tissue.

To generate bone tissue, local progenitor cells are likely to be recruited for proliferation and differentiation into osteoblasts. During the process, cell proliferation is of basic importance for the production of adequate bone-forming cells. However, the proliferative response of osteogenic cells to distraction rates during the distraction phase is not known. To examine the hypothesis that the rate of distraction affects cell proliferation of the regenerating tissue and determines the tissue formed during distraction osteogen-

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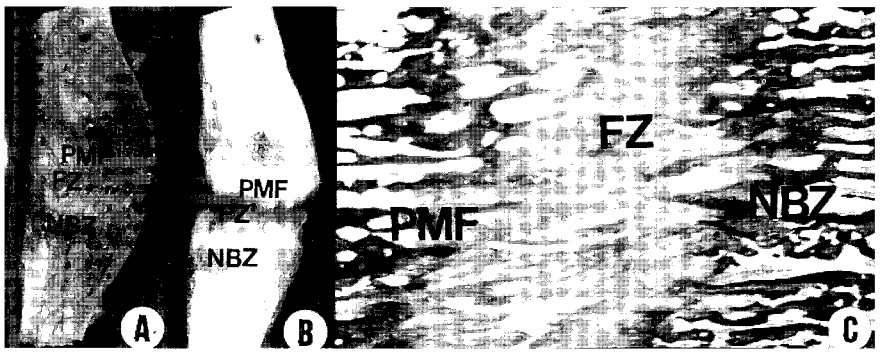

FIG. 1. The three zones of the regenerated tissue as seen in the 0.7 mm/day distraction group. **A:** Gross appearance of the tissue halved longitudinally. **B:** Radiograph of **A.** The central fibrous zone (FZ) is radiolucent, the primary mineralization fronts (PMF) are sclerotic bands adjacent to the radiolucent zone, and the new bone zone (NBZ) has a mesh-like radiolucency close to the compact bone ends. **C:** Histological appearance of the regenerated tissue. Haematoxylin and eosin staining, ×8.

esis, the effect of four different rates of distraction on cell proliferation and on the histology of the regenerated tissue was studied with histological and immunohistochemical methods in a rabbit model of lengthening of the lower limb.

MATERIALS AND METHODS

The experimental protocol was carried out under the control of the Guidelines for Animal Experiments in the Faculty of Medicine, the University of Oxford, and was performed under the Animal License and supervision of the British Home Office.

Animal Model of Lengthening of the Lower Limb

Twenty-five male New Zealand White rabbits (24 weeks old and weighing 3.0-3.5 kg) were distributed into five groups, and a standardised open transverse osteotomy of the right tibial diaphysis was performed. The rabbit was anaesthetised by intramuscular injection of fentanyl citrate (0.2 ml/kg) (Hypnorm; Janssen Animal Heath, High Wycombe, England) and intravenous injection of midazolam (1 mg/kg) (Hypnovel; Roche Products, Welwyn Garden City, England) and locally infiltrated with 0.25% bupivacaine (Marcain; Astra Pharmaceutical, Kings Langley, England). A 4 cm incision was made over the medial aspect of the left tibia, and an Orthofix M100 lengthener (Orthofix SRL, Bussolengo, Italy) was fixed with four stainless-steel screws that were inserted in the tibia. An osteotomy was performed on the tibia at the tibiofibular junction between the two inner screws with use of a handsaw and under saline irrigation. After a 7-day latency period, distraction was initiated at rates of 0 (n = 5) for 2 weeks, 0.3 mm/day (n = 5) for 8 weeks, 0.7 mm/day (n = 6) for 4 weeks, 1.3 mm/day (n = 5) for 2 weeks, and 2.7 mm/day (n = 4) for 1 week, in two steps per day. During the distraction period, the animals were free to bear weight on the involved limb, and weekly radiographs were taken to confirm the lengthening. When lengthening to about 20% (approximately 2 cm) of the original length of the tibia was achieved, the animals were killed.

To investigate the rate of cell proliferation at the different rates of distraction, *in vivo* incorporation of bromodeoxyuridine was applied (1). Bromodeoxyuridine is a thymidine analogue and is incorporated into the DNA during the S phase of the cell cycle. Bromodeoxyuridine (40 mg/kg) (Sigma Chemical, Poole, England) was injected intravenously by a vein in the ear of the rabbit 1 hour before death.

Sample Preparation, Histological Examination, and Immunohistochemistry

The rabbits were killed by an overdose of anaesthesia at the end of the experiment. The central portion of the regenerated tissue in the distraction gap, including 2-5 mm in length of the neighbouring cortical bone, was excised and was halved longitudinally in the median plane with use of a band saw. One-half of the specimen was fixed in 95% ethanol and embedded in glycol methacrylate according to the manufacturer's instructions (BDH Chemicals, Poole, UK); the other half was fixed in 4% paraformaldehyde (pH 7.4) for 24 hours before decalcification in buffered 14.5% EDTA (pH 7.2) for 3-4 weeks at room temperature. The decalcification was confirmed by radiography, and the decalcified specimens were embedded in paraffin wax. Five-micrometer-thick sections were cut with a microtome and placed on slides coated with poly-L-lysine (Sigma Chemical) for histology and immunohistochemistry. For histological examination, the sections were stained routinely with haematoxylin and eosin; Weigert's haematoxylin, alcian blue, and sirius red stain (15) were used to distinguish bone matrix (red) from cartilage matrix (blue). Five-micrometer-thick sections were also cut from tissues embedded in glycol methacrylate, and alkaline phosphatase activity was detected with use of a commercially available kit (Sigma Chemical).

To detect bone-resorbing cells, tartrate-resistant acid phosphatase staining was performed. In brief, paraffin sections were dewaxed and rehydrated through ethanol, washed in Tris-HCl buffer (pH 9.0), and incubated with acetate buffer (0.5% wt/wt sodium acetate and 0.83% vol/vol acetic acid, pH 5.0) for 1 hour before incubation in substrate solutions consisting of naphthol AS-B1 phosphate (15 mg) (Sigma Chemical), sodium tartrate (75 mg), pararosaniline (1 mg), and sodium nitrite (0.8 mg) in acetate buffer (pH 5.0) (26 ml) at 37°C for 1 hour. The sections were rinsed in running water for 5 minutes, with or without counterstaining with haematoxylin and eosin, and mounted in aqueous mounting agent for microscopy (Aquamount; BDH Chemicals).

To reveal the proliferating cells that had incorporated bromodeoxyuridine, paraffin sections were immunostained with anti-bromodeoxyuridine antibody (Bu20A) by the use of an indirect immunoperoxidase method, as previously described (1). The immunostained sections were counterstained with Mayer's haematoxylin and mounted in mountant for microscopy (DPX; BDH Chemicals). The small intestine and thymus are highly proliferative tissues and were used as positive controls for the Bu20A immunostaining. Peripheral nerve and brain tissues are known to be nonproliferative and were used as negative controls for the immunostaining.

Analysis of Bromodeoxyuridine Uptake

For quantitative analysis of the numbers of cells incorporating bromodeoxyuridine, a cell was considered positively stained if brown nuclear staining was visible when it was examined by light microscopy. The positively stained cells were counted, with use of a ×10 eyepiece graticule combined with a ×40 objective lens,

FIG. 2. In the nondistracted control group, the periosteum is thickened and cells positive for anti-bromodeoxyuridine antibody (Bu20A) are seen in the active inner layer of the periosteum as well as in the bone cells on the surfaces of the periosteal callus. Bu20A immunostaining. ×100.

in randomly selected fields of similar morphological appearance within three distinct histological zones, as defined in the Results section. Standard stereological techniques were used. Counting was continued until the cumulative SE of all of the counted fields was less than 10% of the cumulative mean (20). More than 2,000 cells were counted in each section, and for each sample a minimum

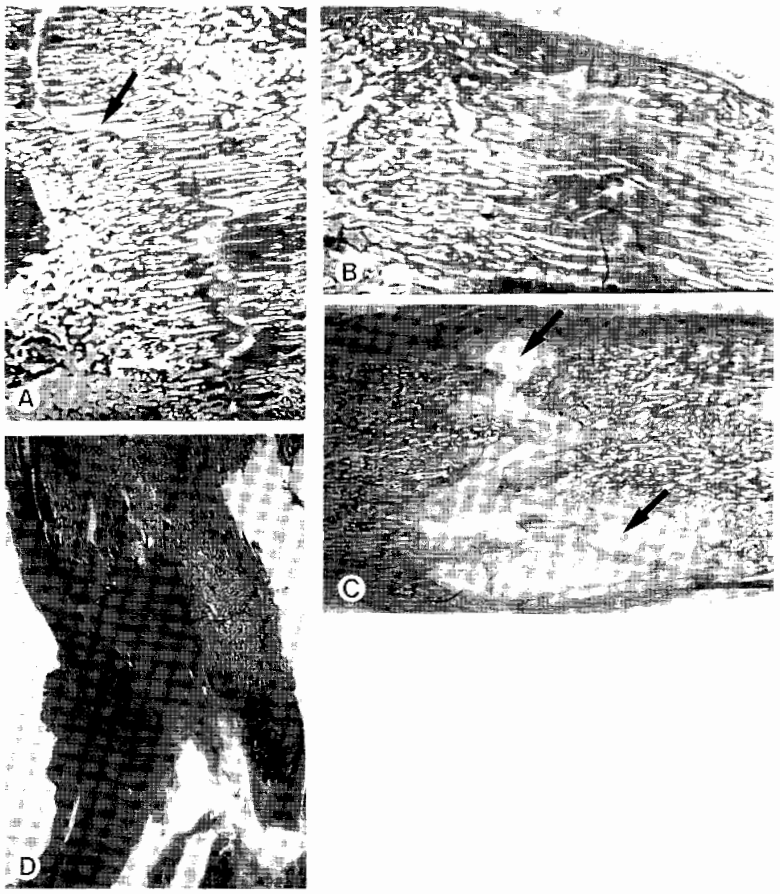

FIG. 3. Morphological appearance of the regenerated tissue. **A:** At a distraction rate of 0.3 mm/day, bone formed parallel to the distraction forces; microfractures caused by distraction can be seen (arrow) (×12). **B:** At a distraction rate of 0.7 mm/day, bone formed mainly by intramembranous ossification. **C:** At a distraction rate of 1.3 mm/day, considerable amounts of cartilage and cyst formation (arrows) were found. **D:** At a distraction rate of 2.7 mm/day, 60-80% of the distraction gap was fibrous tissue and cysts and cartilage islands were often seen. **B-D:** Alcian blue and sirius red staining. ×8.

TABLE 1. *Histological assessments of the regenerated tissue*

Rate of distraction (mm/day)	Cartilage islands	Haemorrhage areas	Cysts	Necrotic areas	Bone remodelling areas
0.3	+/–	–	–	–	+++
0.7	+	+/–	+/–	+/–	+++
1.3	++	++	++	++	++
2.7	++	+++	+++	+++	+

– = absent, + = present in less than 10% of the area of histological sections of the regenerated tissue, ++ = present in 10-30% of the area of histological sections of the regenerated tissue, and +++ = present in more than 30% of the area of histological sections of the regenerated tissue.

of three sections were counted. The positive staining index was defined as the fraction of total cell nuclei that incorporate bromodeoxyuridine. The Bu20A positive staining index values were calculated, together with the mean values and SEMs, for each zone in the different experimental groups. Unpaired one-way analysis of variance was used to determine the level of significance. $P < 0.05$ was regarded as statistically significant.

RESULTS

The constant amount of lengthening of the tibiae resulted in sampling of tissues at various time points for the different distraction groups. This obviously had an effect on the development of tissue in terms of the amount and nature of the tissue formed in the distraction gap and needs to be remembered when the results are considered.

Radiographic and Histological Evaluation of the Regenerated Tissue

Periosteal callus was visible radiographically in the vicinity of the osteotomied bone ends in the control undistracted group at the second postoperative week. Evidence suggesting premature consolidation was seen by radiography in the 0.3 mm/day group (not shown). In all the distraction groups, the distraction gap was seen to consist of three regions at the end of the lengthening period. The three regions, as assessed by histological and radiographic appearances, are shown in Fig. 1 and are defined as follows. (a) The central fibrous zone or interzone, localised in the middle of the distraction gap as a radiolucent area, which gradually enlarged in length as the distraction rates increased. (b) The primary mineralization fronts, which yielded sclerotic bands on the radiographs, lying on both sides adjacent to the central fibrous zone and containing longitudinally arranged, well vascularized collagen bundles that were undergoing mineralization. (c) The peripheral new bone zone, lying between the primary mineralization front and the bone ends of the osteotomy. This zone was seen to consist mainly of mineralised woven and lamellar bone under examination by polarized light microscopy and was relatively radiolucent in radiographs compared with the primary mineralization front. As the distraction rate increased, the length of the fibrous zone increased and that of the new bone zone decreased, but the primary mineralization front remained relatively constant at 2-4 mm in length at all distraction rates (not shown).

Histological Examination and Uptake of Bromodeoxyuridine as Detected by Bu20A Immuuostaining

In the nondistracted control group, the periosteum had become thicker and a large amount of periosteal callus had formed by 2 weeks. Positive Bu20A-labelled cells were seen in the active inner layer of the periosteum as well as in the bone cells on the surfaces of the periosteal callus (Fig. 2).

At the rate of distraction of 0.3 mm/day, the distrac-

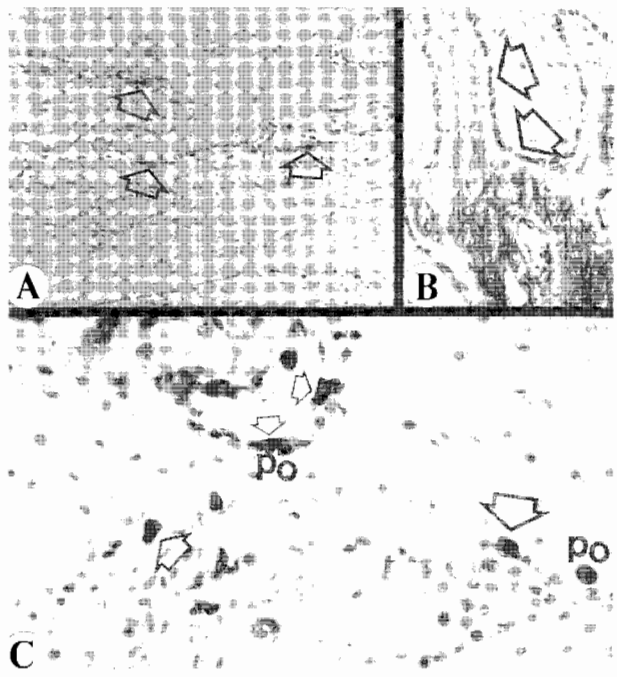

FIG. 4. A: Cells positive for anti-bromodeoxyuridine antibody (Bu20A) were seen close to the bone surfaces (arrows); Bu20A immunostaining, ×100. **B:** The bone surface cells were also positive for alkaline phosphatase (arrows); alkaline phosphatase staining, ×250. **C:** Bu20A-positive cells had round nuclei and were mainly at or near bone surfaces in the new bone zone (arrows); Bu20A immunostaining, ×400. Po = preosteoblast.

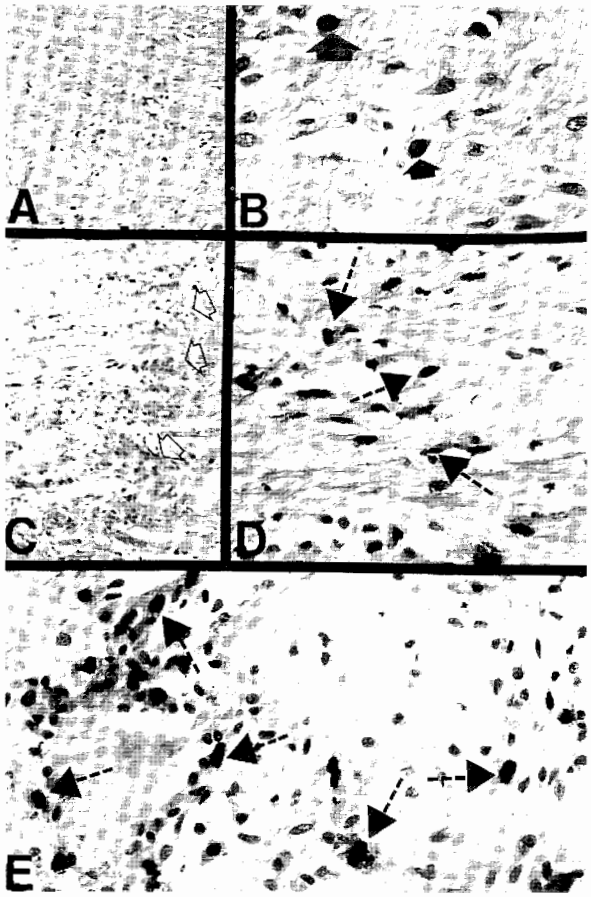

FIG. 5. Anti-bromodeoxyuridine antibody (Bu20A) immunostaining in the 0.7 mm/day group. **A** and **B:** Bu20A-positive cells in the fibrous zone (**A**: ×100 and **B**: ×400). **C:** The new bone was being formed at the junction of the fibrous zone and the primary mineralization front (arrows) (×100). **D:** The greatest number of Bu20A-positive cells (arrows) was observed in the primary mineralization front (×400). **E:** Most of the Bu20A-positive cells in the new bone zone were on or near bone surfaces (arrows) (×400).

tion gap consisted almost entirely of trabecular bone and some fibrocartilaginous tissue. The fibrous zone and the primary mineralization front overlapped in some of the animals. In the new bone zone, consolidation was so advanced that distraction appeared to cause discontinuity in the new bone trabeculae in three of five animals (Fig. 3A). The Bu20A-positive cells were seen mainly near the bone surfaces (Fig. 4A), which were also positive for alkaline phosphatase staining (Fig. 4B). These cells were considered preosteoblasts or osteoblasts because they were polygonal and plump with large round nuclei and a basophilic cytoplasm (Fig. 4C).

When lengthening was performed at distraction rates of 0.7 or 1.3 mm/day, the three zones of the regenerated tissue became more distinct. At 1.3 mm/day, a considerable number of cysts and necrotic areas were found in the regenerated tissue, but these were only occasionally seen in the 0.7 mm/day distraction group (Fig. 3B and C). Sirius red and alcian blue staining revealed moderate amounts of cartilage and cartilage remnants in both groups. In the primary mineralization front, vascular channels were interposed between the collagen bundles, and bone formed parallel to these channels and directed toward both ends of the osteotomy. There were two morphological types of Bu20A-positive cells in the fibrous zone: spindle-shaped, fibroblast-like cells and round osteoblast-like cells (Fig. 5A and B). The greatest positive staining index value for Bu20A was obtained for the primary mineralization front of the 0.7 mm/day group, and most Bu20A-positive cells were at the junction of the primary mineralization front and the fibrous zone (Fig. 5C and D). In this transitional region, the fibrous collagen bundles appeared to be reorganised and bone matrix, to be deposited (Fig. 5C). The Bu20A-positive cells in the new bone zone were close to the bone surfaces (Fig. 5E). General histological assessments of the regenerated tissues are summarised in Table 1.

At a distraction rate of 2.7 mm/day, there was sparse bone formation in the regenerated tissue, and most (60-80%) of the distraction gap was composed of fibrous tissue (Fig. 3D). Compared with distraction at 1.3 mm/day, fewer cartilage areas were seen but larger areas of haemorrhage and necrosis, mainly localised

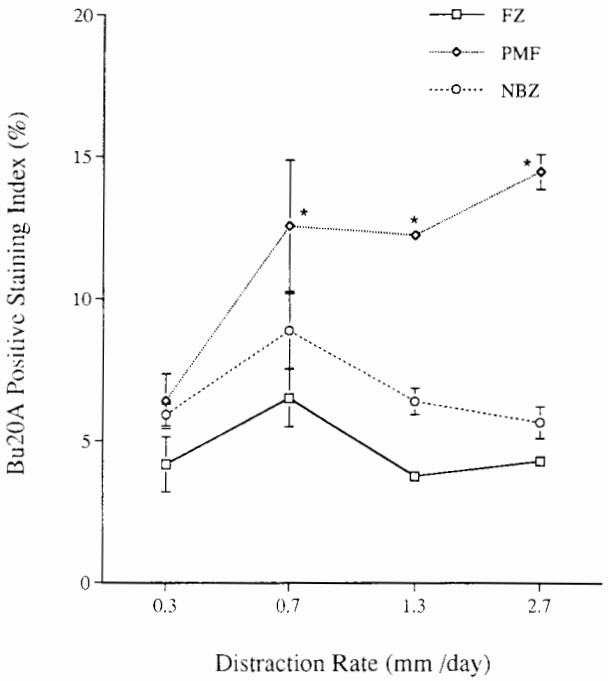

FIG. 6. The anti-bromodeoxyuridine antibody (Bu20A) positive staining index of the different zones of the regenerated tissues at the different distraction rates. There is no statistical difference in the index values with or between the fibrous zones (FZ) and the new bone zones (NBZ) of any distraction group. The values for the primary mineralization fronts (PMF) are the highest (*) among the three regions of all groups except the 0.3 mm/day group. The Bu20A index value for the primary mineralization front increased significantly as the distraction rate increased from 0.3 to 0.7 mm/day (*$p < 0.05$). The index values for the 1.3 and 2.7 mm/day groups are also significantly different from the 0.3 mm/day group (*). Means and SEs are plotted.

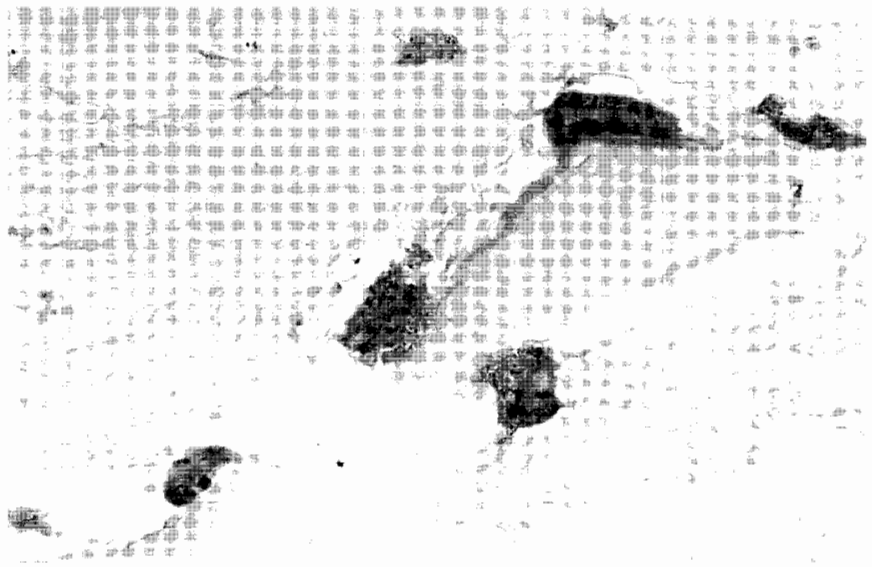

FIG. 7. In all groups, bone-resorbing cells are identified by morphology and by tartrate-resistant acid phosphatase staining. Darkly stained cells are mainly localised in the new bone zone, which is undergoing active remodelling. Tartrate-resistant acid phosphatase staining, ×100.

near the distal end of the regenerated tissue, were observed. The majority of the cells staining positively for Bu20A were observed within the fibrous zone. In the primary mineralization front and the new bone zone, the distribution of these cells was similar to that at distraction rates of 0.7 and 1.3 mm/day.

To quantitate the number of dividing cells in the specific zones, the positive staining index for Bu20A was calculated for each zone at the different distraction rates. In all distraction groups except the 0.3 mm/day group, the index values for the primary mineralization front were significantly higher than those for the fibrous zone and the new bone zone (Fig. 6). No statistical differences were found between the positive staining index values for Bu20A for the fibrous zone and for the new bone zone in any of the distraction groups. The value for Bu20A increased significantly in the primary mineralization front as the distraction rate increased from 0.3 to 0.7 mm/day ($p < 0.05$); this was maintained but did not increase further at the higher distraction rates.

Bone remodelling of the newly formed bone and osteoclastic bone-resorbing cells was identified histologically and by tartrate-resistant acid phosphatase staining in all of the groups (Fig. 7). Cells positive for tartrate-resistant acid phosphatase were mainly localised to the new bone zone distal to the central fibrous region, and there was a general decrease in the number of these cells as the rates of distraction increased.

DISCUSSION

Although distraction osteogenesis has been investigated experimentally with radiographic, morphological, and histological studies, the relevant cellular events in this process are poorly understood. In the present study, we attempted to compare the morphology and cellular response during four rates of distraction. In the clinical situation, the main problems of limb-lengthening are due to the rate, rhythm, and amount of lengthening, and, as evident in our experimental model, the rate of distraction has significant effects on optimal tissue regeneration. To investigate the effects of distraction rates on distraction osteogenesis, the other two variables were kept constant. However, if the amount of lengthening is kept constant and the distraction rate is varied, it is inevitable that the tissue is obtained at different times after the osteotomy. A constant magnitude of lengthening of 20% was used for all of the groups, but the rate of distraction ranged from 0.3 to 2.7 mm/day; therefore, the times the different groups were killed after surgery ranged from 8 weeks (0.3 mm/day) to 1 week (2.7 mm/day). These studies concentrated on the state of the tissues immediately after distraction at the different rates. The higher the rate of lengthening, the shorter the time for regeneration of tissue. However, the tissue regions are morphologically recognisable at all time points. This implies that the whole sequence of regeneration is activated at all distraction rates and indicates that the influence of the distraction rate on regeneration of tissue can be studied by the method used here. Continuous distraction at all the rates used in the present experiments prolongs the process of tissue regeneration (9,18); therefore, it is reasonable to compare the cellular processes occurring in the regenerating tissues at the end of distraction. After distraction at 2.7 mm/day to 20% lengthening followed by a 4 week-consolidation period, cysts and necrosis persist within the regenerated tissue (unpublished observations). This suggests that the necrosis and formation of cysts seen here were not a result of the early

observation times at the higher rates of distraction.

Aronson et al. (3) divided the regenerated tissue into three zones according to morphology and calcium content. The zones were defined as the fibrous interzone (30% of the calcium content of normal bone), the primary mineralization front (40% of the calcium content of normal bone), and the microcolumn formation zone (60% of the calcium content of normal bone). In agreement with Aronson et al., we divided the tissue into the fibrous zone, the primary mineralization front, and the new bone zone. Each of these zones has a distinct morphological appearance as well as a different potential for differentiation and proliferation of cells. The fibrous zone contained spindle-shaped fibroblastic cells that were oriented to the direction of distraction. These cells may have been recruited from the osteoprogenitor cells in the surrounding soft tissues (periosteum, fascia, muscle, and blood vessel) or from bone marrow stromal cells (10-12). The primary mineralization front is a narrow zone, with the highest rate of cell proliferation of the three zones: differentiated osteoblasts start making new bone in this region. The positive staining index values for Bu20A in the fibrous and new bone zones of the distraction groups did not change with the rate of distraction in any of the distraction groups. The results indicate that the rate of cell proliferation of the osteogenic progenitor cells within the fibrous and new bone zones may be constant, and this maximal rate may have been reached soon after the osteotomy. However, the positive staining index value for Bu20A in the primary mineralization front increased significantly ($p < 0.05$) as the rate of distraction increased from 0.3 to 0.7 mm/day. This suggests that the distraction forces applied to the tissues appear to have their greatest effect on cell proliferation in the primary mineralization front. These results indicate that the primary mineralization front may be the region most sensitive to mechanical changes in the regenerated tissue. At distraction rates higher than 0.7 mm/day, the positive staining index value for Bu20A did not increase further in the primary mineralization front, but the areas of cartilage islands, haemorrhages, cyst formation, and necrotic tissues did increase significantly. This indicates that higher rates of distraction (1.3 mm/day or greater) are biologically unfavourable and should be avoided.

Slow distraction rates are sometimes employed clinically because of poor adaptation of the soft tissues. In this model, direct bone formation was achieved at a distraction rate of 0.3 mm/day. In some areas, the regenerated tissue appeared to have healed and fractured; this supports the clinical observation that premature consolidation can occur at slow rates of distraction (18). At a distraction rate of 0.7 mm/day, most of the bone formed in an intramembranous manner, with a few small areas of cartilage and small areas of haemorrhage and cysts, but the overall morphological appearance was satisfactory. Small areas of endochondral ossification were also observed in several previous studies (7,14,16). Ilizarov (9,11) suggested that the presence of cartilage is the result of mechanical instability and found that maximum stability led to direct formation of intramembranous bone, whereas less rigid or unstable fixation led to formation of endochondral bone and fibrous tissue. Others agreed that the instability during the lengthening process could be the main reason for cartilage formation during lengthening (2). A strong, rigid, unilateral fixator, applied in a controlled manner, was used in the present study and the mechanical environment was probably similar at all of the rates of distraction. However, it is possible that at the higher distraction rates, the large daily increment and resultant displacement between the ends of the osteotomy at the time of lengthening disturbed the vascularization and local blood supply to the regenerating tissues.

As a vascular-dependent process, the rapidity of new bone formation during the distraction process requires the maintenance of an adequate blood supply. At distraction rates of 1.3 and 2.7 mm/day, there were considerable numbers of cartilage islands, haemorrhages, cysts, and regions of necrotic tissues in the centre of the regenerated tissue. This may suggest damage to the blood supply of the central tissue. Aronson (4) studied the blood supply of the regenerated tissue by using technetium scintigraphy. The newly formed region of bone showed intense radioactivity, indicating an extensive blood supply, whereas the central fibrous region showed relatively little vascularization by use of this method. At the high rates of distraction (1.3 mm/day or greater) used in the present study, the daily increments may have exceeded the rate of vascularization, with the growth of blood vessels failing to match the extent of lengthening, and thus there was a great reduction in the blood supply to the centre of regenerated tissue. In addition, the excess stretch applied to the tissues may cause spasm and damage of blood vessels and hence haemorrhage and a reduction in the blood supply. Lack of blood supply causes low oxygen tension in the tissue, and under these conditions differentiation of osteoprogenitor cells may be directed toward the chondrogenic pathway to produce cartilage islands (5). Necrosis and formation of cysts also occur as a result of a prolonged lack of blood supply.

At the end of the distraction process, consolidation of the regenerated tissue occurs and the newly formed bone tissue matures and reestablishes a cortex. Aronson (4) reported data from experimental tibial lengthening of more than 125 animals (dogs, rabbits, and rats) and showed that distraction osteogenesis can

provide unlimited new bone formation that remodels at rates of 200-400 μm/day. In the present study, the newly formed bone tissue, produced by both intramembranous and endochondral ossification, underwent active remodelling and resulted in cortical bone. However, the animals were killed at the completion of the lengthening process and consolidation of the regenerated tissue was not complete at the end of the distraction period.

The present study indicates that cell proliferation of bone-forming cells during distraction osteogenesis is affected by the rate of distraction. The rate of cell proliferation cannot be used as the sole criterion to evaluate the distraction osteogenesis process; radiographic and histological assessments of the regenerated tissues are required to give a more complete estimation of the processes at the different rates of distraction. A slow rate of distraction (0.3 mm/day) does not maximally stimulate osteogenic progenitor cells to generate new bone tissue, and a distraction rate of 0.7 mm/day was optimal for cell proliferation and histological characteristics. Whether it is optimal for bone regeneration requires mechanical testing at equivalent time periods after treatment is begun. Higher rates (1.3 mm/day or greater) of distraction caused tissue damage without having any beneficial effect on stimulating proliferation of bone cells and formation of bone in the regenerated tissue.

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