OSTEOGENESIS UNDER CONDITIONS OF TENSILE STRESS

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The morphology of bone formation under the influence of tensile strength arising during limb lengthening by Ilizarov's method has been described in a number of publications [1, 3-6]. Meanwhile the structure of different types of bone tissue formed under those conditions has not yet been investigated, and this was the aim of the study described below.

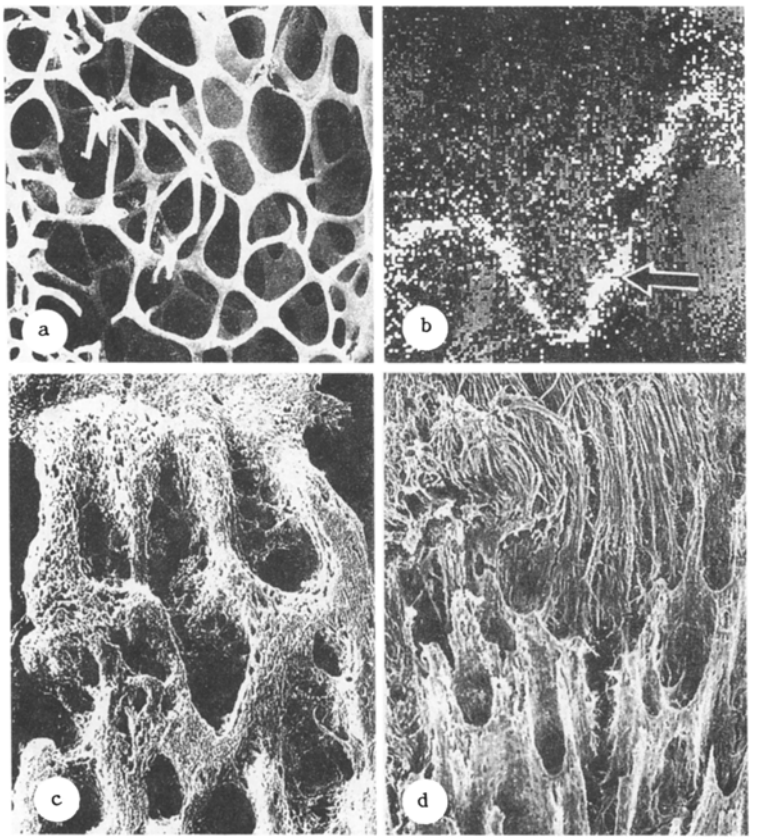

Fig. 1. Characteristics of formation of bone regions and of median layer of distraction-induced regeneration. a) Bone lacunae in medullary canal proximally to fracture line on 7th day of distraction (12th day after operation). Magnification 15, b) sulfated glycosaminoglycans (arrow) in median layer on 7th day of distraction, x-ray picture of calcium and medium. Magnification 6; c) primary osteons formed in areas adjacent to median layer, 7th day of distraction. Magnification 140; d) the same after 21 days of distraction Magnification 50.

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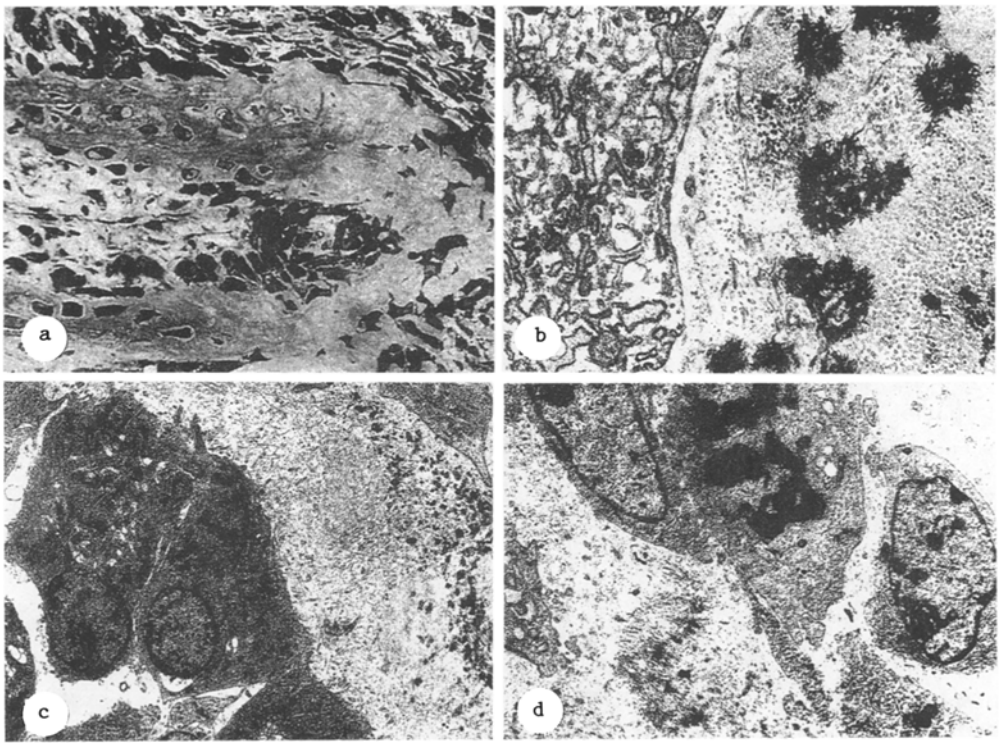

Fig. 2. Morphological features of coarsely fibrous bone tissue. a) Longitudinal section through primary osteon in region adjacent to median layer, on 3rd day of distraction (8th day after operation). PAS reaction, counterstaining with methylene blue. Magnification 250; b) region of osteoblast and zone of calcification in the form of groups of needle-shaped crystals of mineral in collagen fibers on 7th day of distraction. Magnification 20,000; c) osteoblasts on surface of more mature trabecula with zones of calcification around osteocytes on 14th day of distraction. Magnification: 5000; d) mitosis of preosteoblast on surface of newly formed trabecula on 3rd day of distraction. Magnification 8000.

EXPERIMENTAL METHOD

Closed flexion osteoclasia of the tibias was carried out on 60 adult dogs by the method in [2] and 5 days after the operation measured distraction of the bone fragments by Ilizarov's apparatus at the rate of 1-0.75 mm daily, in four applications, began. The animals were taken from the experiment 3 and 5 days after the operation, on the 3rd, 5th, 7th, 14th, 21st, and 28th days of distraction, after 1 and 2 months of subsequent fixation of the limb in the apparatus, and 3 and 6 months after its removal. The regenerating bones were fixed in solutions of paraformaldehyde, glutaraldehyde, and osmium tetroxide and examined in transmission and scanning electron microscopes. Glycoproteins were detected in semithin sections. Calcium and sulfur were determined by x-ray electron-probe microassay, using the method of freeze-substitution.

EXPERIMENTAL RESULTS

The method of flexion osteoclasia disturbs the integrity of a bone but is sparing in relation to the marrow and the tissues surrounding the bone, and the region of operative trauma with this procedure is restricted to a narrow zone around the fracture line. The hematoma formed in this site in the predistraction period becomes partially calcified, as shown by the deposition of needle-shaped crystals of mineral in the platelets, on the fibrin fibers, and in zones containing structural glycoprotein, and it was surrounded by macrophages and fibroblastlike osteogenic cells. Some bone marrow adipocytes also underwent calcification and subsequent phagocytosis. At the end of the predistraction period, growth of capillaries, proliferation of undifferentiated cells, and the formation of fibrils and of collagen fibers were observed; endosteal regeneration took place, connecting the ends of the bone fragments.

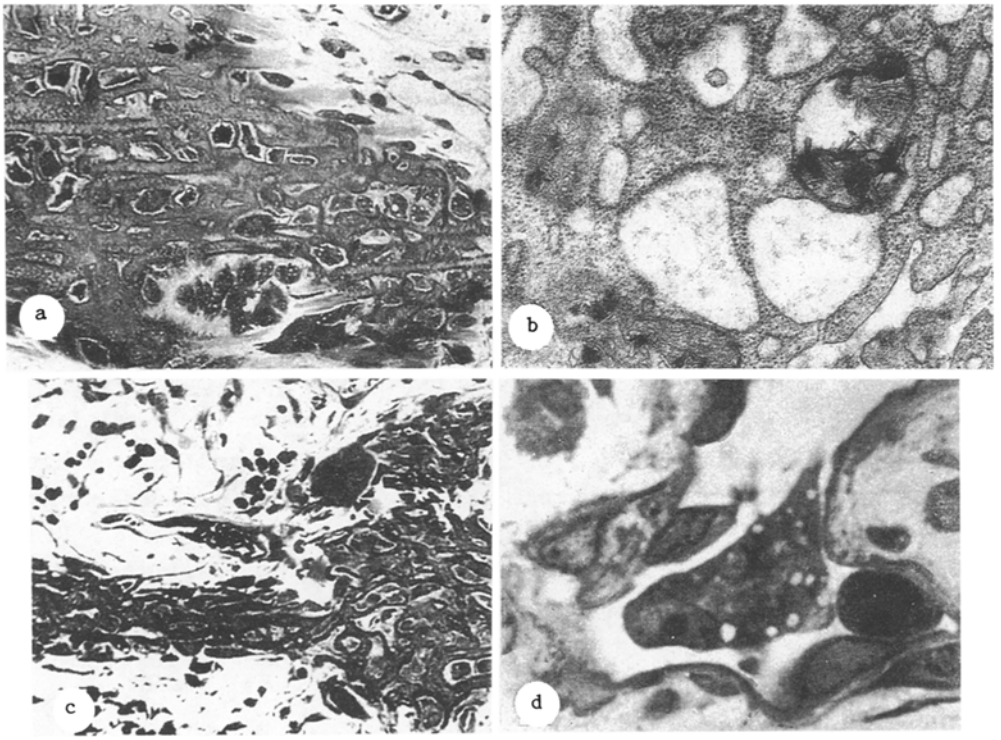

Fig. 3. Structure of reticular bone tissue on 7th day of distraction. a) Area of trabecula with many osteocytes, arranged in rows and in isogenous groups between parallel bundles of collagen fibers. PAS reaction, counterstaining with methylene blue. Magnification 250; b) crystals of mineral in mitochondria of an osteoblast. Magnification 26,000; c) concentration of osteoblasts in widened part of a trabecula. PAS reaction, counterstaining with methylene blue. Magnification 250; d) osteogenic cell in lumen of blood vessel. Stained with methylene blue. Magnification 1500.

The tensile stress arising after the beginning of distraction also induced and supported osteogenesis throughout the period of distraction In the first week bone lacunae filled the medullary canal with a dense fine-looped network proximally and distally to the fracture line (Fig. 1a) and, spreading into the diastasis, formed two bony regions of regeneration, separated by a median layer containing sulfated glycosaminoglycans (Fig. 1b), longitudinally oriented capillaries, bundles of collagen fibers, and many undifferentiated cells. In parts of the regenerating bone adjacent to the median layer, the formation of primary osteons from coarsely fibrous and reticular bone tissue was observed unusually early, namely on the 3rd day of distraction (8th day after the operation), and this continued until the end of the period of distraction (Fig. 1c, d).

Coarsely fibrous bone tissue formed fibroblastlike perivascular cells of polygonal shape with numerous vacuoles of the Golgi complex and lipid cytoplasmic outgrowths, lying between parallel rows of collagen fibers, the degree of calcification of which increased with an increase in the distance from the median layer (Fig. 2a). Zones of calcification in the form of star-shaped groups of needle-shaped crystals (Fig. 2b), arranged chaotically along the fibers, gradually merged, and in more mature areas of the trabeculae they formed a continuous front of mineralization around the osteocytes (Fig. 2c). In the perivascular spaces and at the periphery of the trabeculae patterns of mitosis were often observed (Fig. 2d) with the formation of concentrations of osteoblasts, which, surrounded by areas of collagen fibers with zones of cacification, were converted into osteocyes, arranged in parallel rows along the trabeculae. The osteocytes preserved features of biosynthetic activity with a gradual reduction of secretory activity, as shown by the presence of numerous cisterns of rough endoplasmic reticulum in them and accumulation of glycoprotein in the vacuoles of the Golgi complex.

In the reticular (fascicular) bone tissue a network of interwoven collagen fibers formed thick, longitudinally arranged bundles toward the end of the 1st week of distraction, between which lay osteocytes in parallel rows and isogenous groups (Fig. 3a). They contained greatly dilated cisterns of the rough endoplasmic reticulum and swollen mitochondria with crystals of mineral (Fig. 3b) and they belonged to a population of short-living cells, as shown by the appearance of destructive changes in them after calcification of the matrix. Areas with dying osteocytes were surrounded by osteoblasts of reticular or coarsely fibrous

bone tissue, or they were subjected to osteoclastic resorption, with in growth of capillaries into the newly formed cavities. In the latter case the trabeculae acquired their characteristic shape (Fig. 3c). Concentrations of osteoblasts, surrounded by a network of calcified collagen fibers, in the dilated and least calcified areas, and were converted into osteocytes and quickly died. Thanks to the presence of natural intracellular markers, such as vacuolated mitochondria with crystals of mineral, the osteogenic cells of the reticular bone tissue could also be identified, not only when in the composition of the endothelium, but also when in the lumen of blood vessels (Fig. 3d), evidence that they belonged to the population of freely circulating cells. Possessing pluripotent properties, depending on their microenvironment and on the concrete biomechanical conditions, and especially under the influence of tensile stress, they were able to realize their fibrogenic, osteogenic, and chondrogenic potential.

Toward the end of the 1st month of fixation the median layer was replaced by coarsely fibrous bone tissue, but by the end of the 2nd month, a cortical lamina was formed, in which secondary osteons of laminar bone tissue developed, completely replacing the woven bone 6 months after removal of the apparatus. By this time, the structure of the regenerating bone tissue was similar to that of intact bone.

These investigations showed that three types of bone tissue took part in the formation of the regenerating bone during distraction of the lengthened limb: woven (coarsely fibrous), reticular (fascicular), and laminar, of which the first two were provisional, rapidly renewed bone, whose volume increased not only through appositional growth, but also interstitially. Their new formation proximally and distally to the median layer facilitated growth of the primary osteons formed in this region, and, consequently, of the whole regenerating bone.

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