HISTOGENESIS OF SKELETOGENIC TISSUE DURING BONE REGENERATION UNDER DISTRACTION

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The ability of mesenchymal cells to form bone substance is not itself an exceptional phenomenon in biology. For instance, the mesenchymal cambium remains capable, throughout human life, of differentiating into blood cells in any organ, and this is frequently observed, for example, in the various leukemias [4].

Recent investigations have shown that the high potential capacity of skeletogenic tissue to differentiate may be manifested to fully during application of biomechanical factors associated with the use of modern special kinds of apparatus.

Great difficulties have been noted in the past during attempts to determine the capacity of skeletogenic tissue for plastic repair during fracture healing [4]. Methods of treatment of bone injuries used until recently did not enable the high potential regenerative capacity of skeletogenic tissue to be utilized to the full [2].

High technological orthopedic apparatuses easily controlled by the surgeon, which have appeared in recent years, namely devices for fixing bone fragments [1, 2, 5], enable the most widely different biomechancial conditions needed for osteogenesis or chondrogenesis to be created and have enabled the morphogenesis of regenerating bone to be studied in convenient experimental models.

The aim of this investigation was to study histogenesis of regenerating bone on a model of bone lengthening during distraction osteosynthesis as described by G. A. Ilizarov.

EXPERIMENTAL METHOD

To obtain a model of bone lengthening of growth type, oblique transperiosteal osteotomy was performed in the proximal metadiaphysis of the tibia. Experiments were carried out (V. P. Shtin and G. A. Onoprienko) on adult mongrel dogs. Distraction with G. A. Ilizarov's apparatus began in all animals 7 days after oblique transperiosteal osteotomy. Distraction was at the rate of 1 mm daily and continued for 7, 14, and 21 days. To study histogenesis of regenerating bone under distraction, material was studied histologically (sections stained with hematoxylin and eosin and by Van Gieson's method) and by electron microscopy. Pieces of regenerating tissue were fixed and embedded in the usual way. Ultrathin sections from undecalcified bone tissue were cut on an LKB-4800 Ultratome, counterstained with lead citrate, and examined in the JEM-7A electron microscope.

EXPERIMENTAL RESULTS

Histologic investigation 1 week after osteotomy, during which the bone fragments were immobilized, showed that by the 7th day before the beginning of distraction, besides residual post-traumatic necrobiotic changes, loose fibrous connective tissue with bundles of collagen fibers running in different directions, and containing small tissue cysts and single capillaries were observed in the narrow (under 300 µ) gap between the fragments. No main blood vessels were present in this zone.

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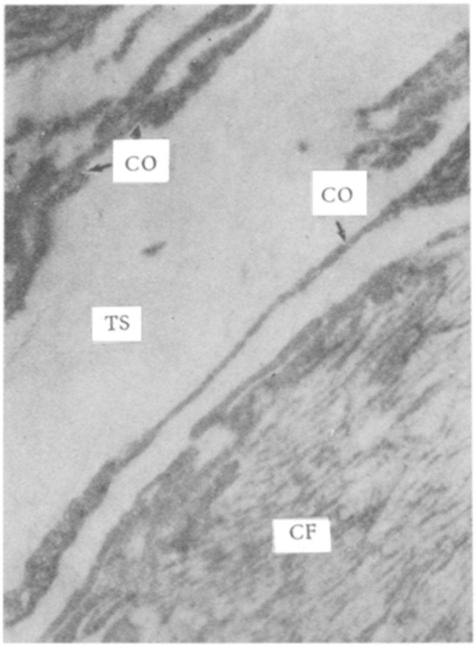

Fig. 1

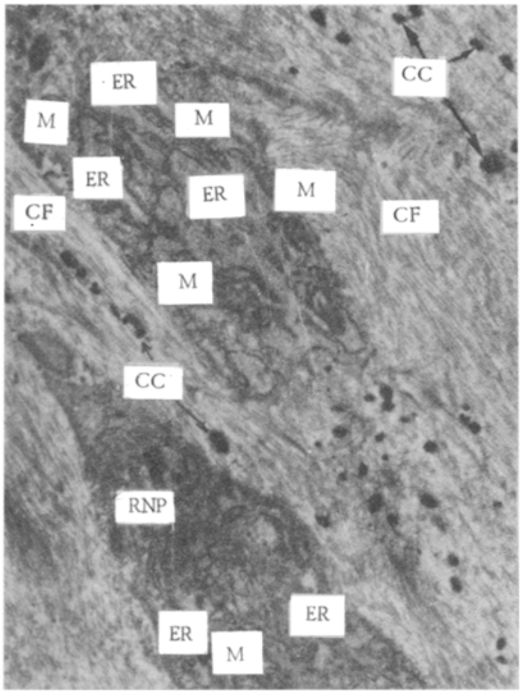

Fig. 2

Fig. 1. Tissue space lined with flattened outgrowths of fibroblasts. CO) Cytoplasmic outgrowths of fibroblasts; CF) collagen fibrils; TS) tissue spaces. 26,000 ×.

Fig. 2. Initial stages of mineralization of peripheral parts of growth plate. RNP) Ribonucleoproteins; ER) endoplasmic reticulum; CC) accumlulations of calcium crystals; M) mitochondria. 7800 ×.

Toward the end of the first week of distraction and the second week after osteotomy, a longitudinal arrangement of the bundles of collagen fibers was seen in the gap between the fragments, accompanied by a band of osteogenic tissue with longitudinally arranged vessels and elongated bony trabeculae near the cut ends of the distracted bone fragments.

The subsequent course of reparative osteogenesis under distraction for a period of between 1 week and 1 month was characterized by an increase in the gap between the fragments, which was filled with regenerating tissue, while in its middle part there was a band of fibrous tissue with longitudinally arranged bundles of collagen fibers, on the basis of which long bony trabeculae were forming and maturing in the direction of the increasingly distracted fragments. This connective-tissue band resembled a growth plate, and the regenerating tissue between the fragments was made up of zones of bone structures differing in maturity. A similar picture is observed in the apophysis during growth of the tibia [3].

Electron-microscopically, on the 7th, 14th, and 21st days of distraction, definite morphological features evidently associated with their functional state were found in fibroblasts of the connective-tissue growth plate, lying among collagen fibrils about 35 nm in diameter and cross-striation of 30-35 nm, arranged longitudinally. On this basis the fibroblasts were divided into three types. The first type consisted of actively synthesizing fibroblasts with a well-developed endoplasmic reticulum. This is morphological evidence that intensive synthesis of protein of collagen type is taking place in the cells. They were distributed mainly in the central zones of the growth plate. The second type consisted of differentiating fibroblasts, already possessing all the necessary cytoplasmic organelles. The third type of fibroblasts had a poorly developed endoplasmic reticulum. It must also be noted that not only the fibrillary structures and fibroblast-like cells were arranged longitudinally in accordance with the direction of traction, but so also were their cytoplasmic organelles. This ultrastructural characteristic of the connective-tissue layer between the fragments was of absolutely identical type throughout the period of distraction. The conditions of the blood flow in the growth plate also demand attention. Along the collagen fibrils and among the fibroblasts, erythrocytes could be seen, arranged either singly or in groups, in the form of columns, and in the immediate vicinity of the cytoplasmic membrane of the

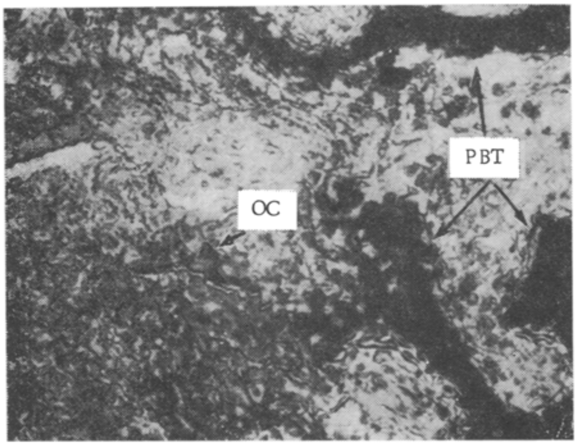

Fig. 3. Zone of osteogenesis, newly formed primitive bony trabeculae. OC) Osteoblast cells; PBT) newly formed primitive bony trabeculae. Semithin section. Stained with azure II. Magnification 32×.

fibroblasts, together with tissue cysts and spaces. This picture must evidently be regarded as an open blood flow. Fibroblasts of the second type gradually began to line the tissue spaces with their thin flattened outgrowths (Fig. 1), and in all probability this marked the initial stages of formation of capillaries, which were not yet completely formed in the interior of the connective-tissue plate. However, at its edges, where bundles of collagen fibrils merge with bony trabeculae, closed blood capillaries of sinusoid type were formed. This description serves as a basis for the view that the cells described above are the source not only for osteogenesis, but also for angiogenesis.

The presence of osteoblasts, also in an active functional state, and of undifferentiated preosteoblasts was observed later at the periphery of the connective-tissue growth plate. In the immediate vicinity of the connective-tissue growth plate unmineralized osteoid containing longitudinal bundles of collagen fibrils about 40 nm thick with cross striation of 40-50 nm could be seen. Along the bundles of collagen fibers were cells of osteoblast and fibroblast types as well as preosteoblasts, so that their long axis was parallel to the course of the collagen fibrils. Osteoblasts were characterized by the presence of a well-developed rough endoplasmic reticulum with parallel arrangement of the tubules and lamellar complex, which was localized in the perinuclear zone. In the cytoplasm of the preosteoblasts there were many free ribosomes and polysomes. The rough endoplasmic reticulum consisted of single flattened tubules. The ultrastructure of the fibroblasts was the same as that of fibroblasts located in the growth plate. In areas of regenerating tissue more distant from the fibrous connective-tissue growth plate the first traces of mineralization of collagen fibrils were seen in the form of small concentrations of single hydroxyapatite crystals (Fig. 2). Collagen fibrils in mineralized osteoid were somewhat thicker, 40-50 nm in diameter, and their cross striation measured about 60 nm. Only osteoblasts were found here, arranged in rows along the long axis of the collagen fibrils (Fig. 3). These osteoblasts were irregularly cubical in shape, and densely packed one against another. The cells were joined together in such a way that evagination of the cytoplasmic membrane of one cell formed on invagination of another. Osteoblasts in the zone of mineralized osteoid were characterized by the presence of a well-developed endoplasmic reticulum, consisting of a system of greatly dilated cisterns and lacunae, occupying the whole cytoplasm. The lamellar complex also underwent marked development and occupied the perinuclear zone.

The investigation of histogenesis of bone tissue regenerating under distraction, under the conditions of G. A. Ilizarov's stable distraction osteosynthesis, thus showed that filling of the distraction diastasis with newly formed bone takes place through continuous growth of bone tissue. Osteogenesis under these circumstances is based on a constantly present fibrous connective-tissue growth plate, located in the middle part of the regenerating tissue filling the diastasis. Besides continuously repeated osteogenesis, continuous angiogenesis also takes place in this plate. The source of its formation is mainly the endosteum of the opposed fragments. Considering that the fibrous connective tissue of the growth plate contains actively functioning fibroblasts, it must be postulated that its preservation is due to the stimulating effect of distraction on the process of fibrilogenesis. Intensified fibrilogenesis leads to preservation of fibrous tissue in the diastasis, despite a continuing process of mineralization.

The special features of histogenesis of bone and connective tissue in the connective-tissue zone of regenerating bone between the fragments, revealed by this "distraction" model, suggest that their histogenesis is common in nature. Osteogenesis and angiogenesis in bone tissue regenerating under distraction were effected when only fibroblasts were present. No other cell source of histogenesis could be found. The process of osteogenesis and angiogenesis in a so-called connective-tissue growth plate of bone regenerating under distraction during lengthening by G. A. Ilizarov's technique can thus take place under strictly definite, controlled, and graded biomechanical conditions as provided by modern special orthopedic apparatuses. They create the necessary control for cell proliferation and growth without any marked destructive and necrobiotic changes in the distracted tissues. This phenomenon demands a profound knowledge of the physiology of connective tissue as well as high skill on the part of the surgeon who is actively intervening in the repair process.

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