Osteogenesis and Angiogenesis during Distraction Osteosynthesis

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The volume-spatial organization of osseous tissue and the microarchitectonics of distraction bone regenerating regions of 80 dogs with crus elongation performed after Ilizarov are studied at different stages using the methods of light microscopy, transmission and scanning electron microscopy, and x-ray electron probe microanalysis. It is shown that the rarefaction the interstitial spaces resulting from controlled distension of the bone fragments exerts a stimulatory and orienting effect on angiogenesis. The vascular loops from the proximal and distal fragments and from the paraosseous tissue grow in opposite directions, meet, and intrude into the opposite fragments. Primary osteons develop around these loops. The rapid elongation of osteons insure the growth of the regenerating bone tissue in the desired longitudinal direction.

Key Words: *osteogenesis; angiogenesis; limb elongation*

The high efficacy of the modalities of treatment established under the supervision of G. A. Ilizarov is well known. At the same time, the data concerning morphological peculiarities of the osseous tissue and blood vessels in the regions of distraction bone regeneration are scant and controversial [1-4].

The goal of this work was to study the volume-spatial specific features of the bone tissue and the vessels of the microcirculatory bed in bone regenerates formed during limb elongation after the method of Ilizarov.

MATERIALS AND METHODS

Eighty adult dogs underwent closed flexion osteoclasia of the tibiae. Five days later a distraction (controlled distension of the bone fragments) was started using the device developed by Ilizarov, at a daily rate of 0.75-1 mm, in 4 procedures per day. Animals were taken 5 days postoperation, 3, 5, 7, 14, 21, 28, and 42 days after the start of distraction, 1 and 2 months after subsequent fixation of the limb in the apparatus, and 3 and 6 months after removal of the apparatus. The tibial epimetaphyseal regions of 18 1-6-month-old puppies were also studied. Microarchitectonic and morphofunctional peculiarities of the osseous tissue and blood vessels in the regions of bone regeneration were studied on cleared unstained preparations and on semithin sections stained with Schiff reagent and methylene blue. The study also included transmission electron microscopy of ultrathin sections and scanning electron microscopy of polished bone sections, corrosion preparations, and injectional corrosion replicas of blood vessels [5]. The distribution and concentration of calcium in the bone sections were recorded using an x-ray electron probe microanalyzer.

RESULTS

Analysis of the bone marrow channels in the fragments adjacent to the fracture line five days postoperation reveals the formation of a fine-mesh

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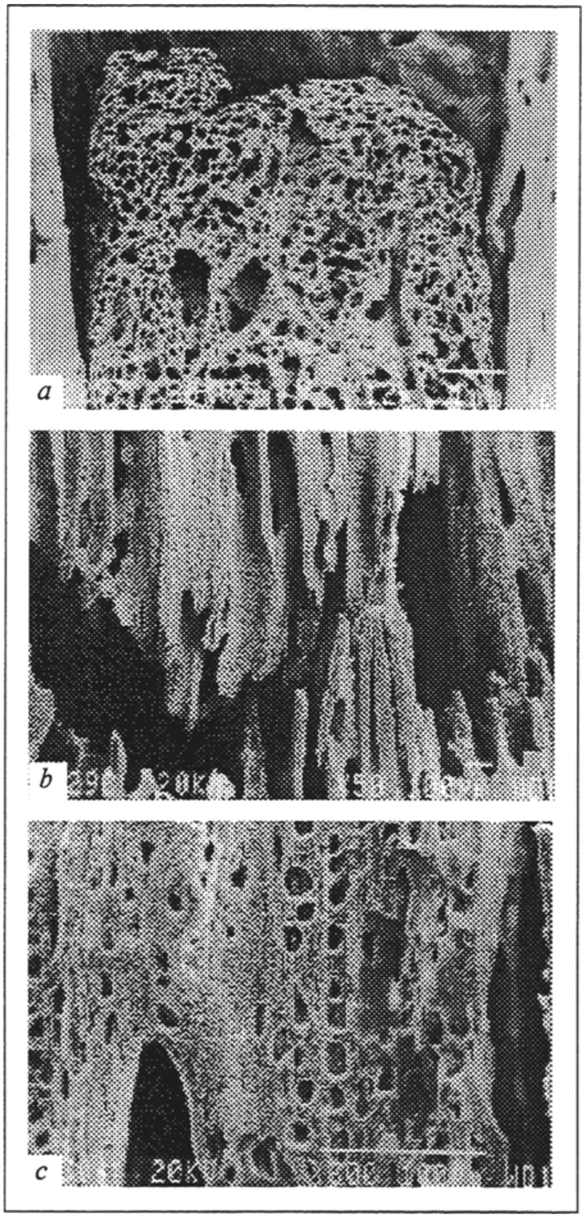

Fig. 1. Volume—spatial peculiarities of bone tissue in the area of regeneration. *a*) closed bone lacunae in the bone marrow canal of one of the fragments. ×9; *b*) primary osteons invading the middle layer of the region of regeneration. ×30; *c*) osteocyte—formed lacunae arranged in longitudinal layers in primary osteons. ×220. Scanograms of corrosion preparations on the 14th day of distraction.

network of bone-osteoid trabeculae and the growth of sinusoids that are surrounded by numerous poorly differentiated cells. The lumens of the *de novo* forming capillary terminals often exhibit freely circulating osteogenic cells containing natural intracellular markers, namely vacuolized mitochondria with mineral crystals in the matrix. At this time the primary bone-osteoid fusion containing 2-4% calcium develops.

Beginning from the first day of distraction, the area of regeneration becomes considerably enlarged, mostly on account of endosteal osteogenesis. The regeneration involves not only the diastasis, but also the bone marrow channel of the fragments over a considerable extent. In this region and in the diastasis of fractured pieces bone lacunae are formed which, under conditions of stable fixation and the absence of external mechanical distension, close up and acquire a round-ellipsoid shape (Fig. 1, *a*). In the region of distension the spheroid lacunae along the fracture line become transformed into longitudinally oriented cylindrical primary osteons (Fig. 1, *b*). Their apexes are open and grow toward each other, thus providing for osteogenesis in the desired longitudinal direction and rapid elongation of the regenerating bone. The length of primary osteons increases by 300-500 μ per day and is a function of two antagonistic processes, namely osteogenesis at the top and resorption at the base, where they come in contact with the bone lacunae. Rapid longitudinal growth of the primary osteons is insured by the formation of osseous tissue with a peculiar structure which is capable of both appositional and interstitial growth. Within this tissue numerous osteocytes with hypertrophized structures of the protein-synthesizing apparatus form longitudinal sheets resembling the chondrocytes of the epiphyseal plate in the puppies (Fig. 1, *c*).

Thus, as a result of distraction the regenerating bone acquires a zonal type of structure. On both sides of the ends of tissue fragments lies a region of round bone lacunae adjacent to the zone of primary osteons. This zone is divided into proximal and distal parts by a weakly calcified middle layer that includes the growing osteoid apexes of the osteons and bundles of collagen fibers of the developing fibrous-granulation tissue (Fig. 2, *a*). The abolishment of distraction arrests the longitudinal growth, the zone of primary osteons shrinks and is ultimately completely resorbed (Fig. 2, *b*, *c*), and the weakly calcified layer is replaced by bone tissue through a much slower appositional growth of the small spheroid bone lacunae.

Distraction also causes a series of microarchitectonic peculiarities in the regenerating tissue. The growth rate of blood vessels often exceeds the rate of distraction (0.75-1 mm per day), as can be judged from their convoluted, spiral shape. It is known from the literature [6] that as a rule the maximal rate of capillary invasion into the regenerating tissue does not exceed 0.1 mm a day.

Starting from the first week of distraction, arteriolar, venular, and capillary loops from the haversian canals of primary osteons start to invade the

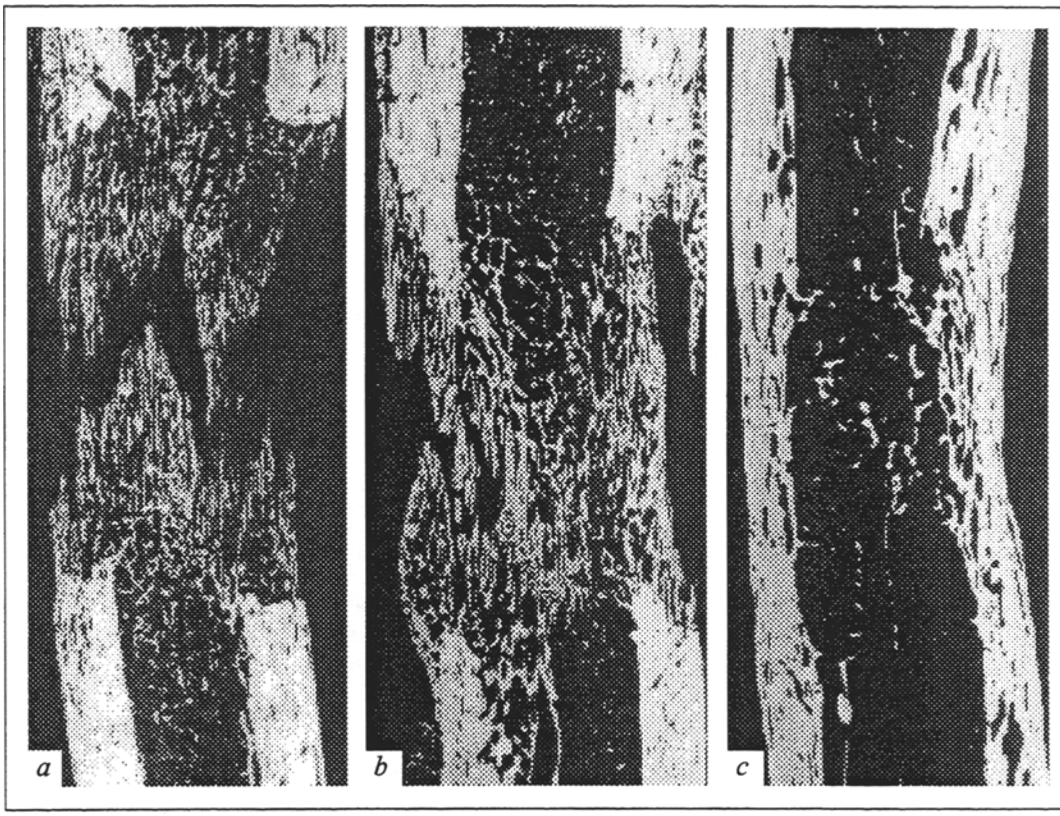

Fig. 2. Structural peculiarities of distraction bone regeneration regions at various stages of limb elongation. *a*) zonal structure of regenerating tissue on the 28th day of distraction; *b*) replacement of the middle layer by bone tissue and consolidation of the proximal and distal parts on the 30th day of fixation in the apparatus (63rd day postoperation); *c*) formation of the cortical layer and of the bone marrow canal in the newly formed region of diaphysis 6 months after removal of the apparatus (264th day postoperation). ×4. Scanograms of bone tissue sections. Image produced in reflected electrons.

middle layer. From the periosteum at a right angle to it arterioles intrude into the regenerating region, thus organizing a united system of blood flow of the medullar and periosteal zones. The development of arteriole-venule loops also takes place, their apexes giving rise to capillary terminals. The middle layer is traversed in the vertical direction by elongated and thin parts of the lateral branches of the nutrient artery which provide for the continuity of the vascular bed of the distal and proximal fragments of the regenerating region.

The most intensive capillarogenesis goes on near the inner surface of the cortical plate that is being formed in the regenerating tissue (Fig. 3, *a*). Within this region the lateral branches of the nutrient artery divide into small, richly branched arterioles that give rise to capillaries. At the points of arteriolar branchings (trifurcations) form characteristic accumulations of triangular, flat cisternae which function as some kind of vascular collectors. The developing interarteriolar anastomoses act as buffer structures and thus participate in the distribution of the blood flow. Capillary terminals, in the form of blunt, sharp-ended, or branched processes, invade the middle layer. Departing from the terminal arteriolar branches and forming anastomoses, they give rise to narrow capillary loops stretching in the oblique-transverse direction across the middle layer. The terminals formed by the lateral branchings of the nutrient artery come together to form broader loops with a longitudinal orientation.

The haversian canals of the periosteal osteons contain mostly vessels of the venule type that are connected by circular parallel vessels in the developing Volkmann's canals (Fig. 3, *b*).

In the periosteum surrounding the middle layer the newly formed vessels have a predominantly circular orientation and form loops disposed in an oblique-transverse direction. Certain vessels acquire a ribbonlike form. Unusual types of vascular interaction occur, whereby growing capillary terminals push through larger vessels or twine around them in a spiral (Fig. 3, *c*).

On the periosteal surface multiple perforations of the cortical plate are seen, through which the newly formed arterial and venular branches of predominantly longitudinal orientation penetrate in both directions, i.e., into the regenerating region and from it into the surrounding soft tissues (Fig.

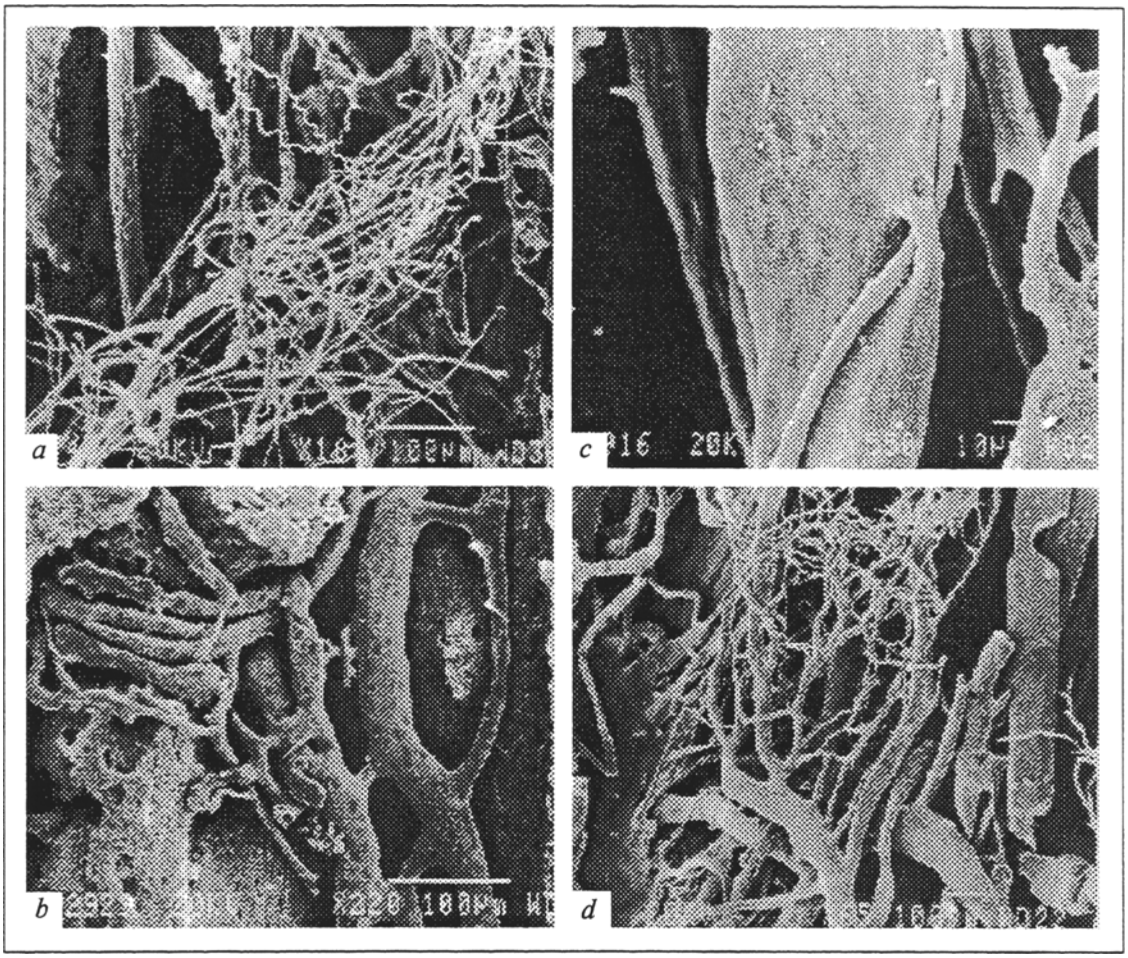

Fig. 3. Specific features of microarchitectonics in the distraction bone regeneration region. *a*) microarchitectonics at the inner surface of the forming cortical plate. ×130; *b*) spatial organization of the microvascular bed in the haversian canals of the periosteal osteons (the outer part of the bone matrix has been removed). ×160; *c*) capillary terminal penetrating a flat venule on the periosteal surface of the regeneration region. ×400; *d*) terminal vascular bed of a fragment of the anterior tibial muscle adjacent to the periosteal surface of the regenerating tissue. ×50. Scanograms of corrosion replicas of blood vessels on the 42nd day of distraction.

3, *d*). Closer to the ends of the bone fragments on the periosteal surface we find large, interlacing, circular vessel bundles reappearing side by side with the longitudinal vessels.

The presence of multiple circular vessels invading the regenerating region and paraosseous tissues and the high growth rate of the capillary terminals show that during distraction, within the tissue structures of an elongated limb there arise not only forces of distension that stimulate fibrillogenesis and cell proliferation and differentiation [7], but also concomitant forces of rarefaction that are connected with the distraction-associated widening of the interstitial spaces. The latter have a "sucking-in" effect on the growing capillary terminals, thus stimulating angiogenesis in the desired direction. This feature is being exploited in clinical practice at the Russian Science Center "Reconstruction Traumatology and Orthopedics" in the treatment not only of the locomotor apparatus, but also of injuries to the spine and spinal cord.

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