ELECTRON-AUTORADIOGRAPHIC STUDY OF BONE FORMATION DURING DISTRACTION OSTEOSYNTHESIS

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To replace defects in bone tissue the method of graded distraction osteosynthesis, suggested by Ilizarov in 1967 [3], is being used on an ever-increasing scale. Its essential difference from other methods of replacement of bone tissue defects is that no graft of any kind is introduced from outside into the wound. Graded distraction osteosynthesis is based on gradual separation of the opposed ends of the osteotomized bone, in the process of which the resulting diastasis is filled with regenerating tissue, which subsequently is transformed into bone tissue.

Initially the region of the diastasis is filled with young connective tissue consisting of fibroblast-like cells, longitudinally oriented collagen fibers, and capillaries; later, foci of osteoid tissue and osteoblasts appear, small bony trabeculae are formed, and later still, normal bone tissue [2, 5]. The large number of capillaries in the regenerating tissue is noteworthy [1, 2, 4, 5]. However, it is not yet clear whether the role of the blood vessels is limited solely to the supplying of nutrients to the regenerating tissue or whether they play an active role in the intimate mechanisms of osteogenesis also.

A contribution to the solution of this important problem can be made by the use of electron-microscopic autoradiography, for with this technique not only can different cells of the regenerating tissue be identified more exactly, but those which are distinguished by more intensive DNA synthesis and, consequently, which are proliferating cells of this tissue, can also be established.

In the investigation described below bone formation was studied during replacement of bone tissue defects by graded distraction osteosynthesis in 12 patients with open fractures of the tibia, accompanied by extensive soft tissue damage.

EXPERIMENTAL METHOD

Osteotomy was performed subperiosteally in the region of the proximal epimetaphysis of the tibia, which is rich in spongy substance. To monitor the course of regeneration, biopsy was performed on the regenerating tissue immediately after the end of distraction and after removal of the external fixation (Ilizarov's apparatus), when the picture of normal regenerating bone was identified roentgenologically.

Part of the biopsy specimen was fixed in neutral formalin and embedded in paraffin wax; sections were stained with hematoxylin and eosin and by Van Gieson's method. The rest of the material was examined by electron-microscopic autoradiography. For this purpose, pieces measuring 1 mm^3 were incubated for 1.5 h at 37°C in medium 199 with [^{3}H]thymidine (specific activity 21.6 Ci/mmole) in a dose of 10 μCi/ml, and also with [^{3}H]uridine (specific radioactivity 26.0 Ci/mmole) in a dose of 100 μCi/ml. After incubation the fragments were washed with cold phosphate buffer, pH 7.4. The material was fixed in 2.5% glutaraldehyde solution and 1% OsO_4 solution and embedded in Epon. Semithin sections were first investigated by light-microscopic autoradiography. Depending on the results of this analysis, regions for cutting ultrathin

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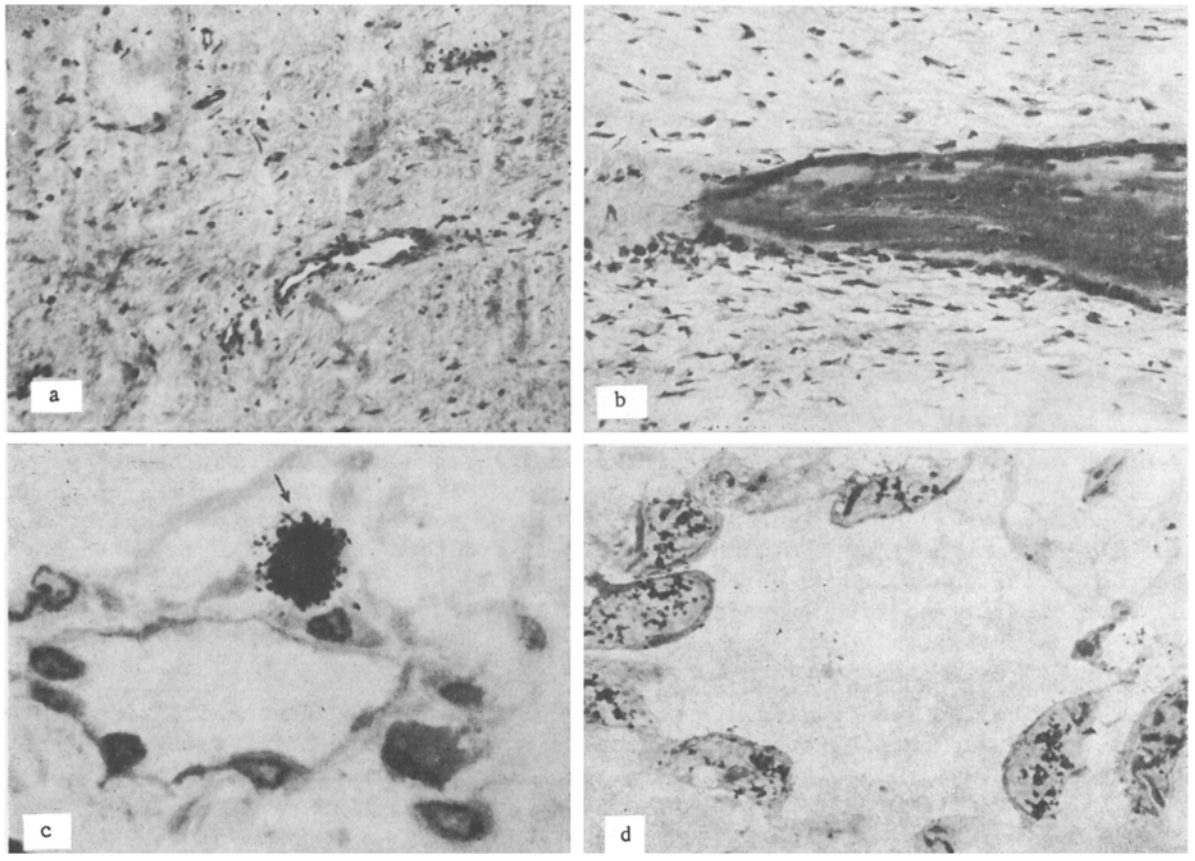

Fig. 1. Regenerating tissue in the early stage of formation: a) large number of blood vessels and single fibroblasts, distributed among mass of collagen fibers. Hematoxylin—eosin, 160 ×; b) formation of bony trabecula, with osteoblasts arranged around it. Hematoxylin—eosin, 260 ×; c) DNA synthesis in a cell in close proximity to a blood vessel (arrow). Semithin section. Toluidine blue, 1000 ×; d) RNA synthesis (black grains of silver) in cell nuclei of vessel wall, 10,000 ×.

sections were selected in the semithin sections. Electron-microscopic autoradiographs were prepared by the method described previously [6, 7].

EXPERIMENTAL RESULTS

Regenerating tissue for investigation, removed immediately after the end of distraction, was firmly elastic to the touch and grayish-white in color. Microscopically it consisted of fibrous connective tissue, represented by thin collagen fibers oriented longitudinally, numerous small blood vessels, and single fibroblasts, which were distributed among the mass of collagen fibers (Fig. 1a). Small areas of newly formed bone were found among the collagen fibers and fibroblasts. They consisted of trabeculae of different sizes, some of which were composed of coarse collagen fibers, changing into osteoid and bone tissue, whereas others were already formed bone trabeculae. Many osteoblasts could be seen around these foci of osteogenesis (Fig. 1b).

In the late stages after the end of distraction, when roentgenologically the regenerating tissue now consisted of normal bone, on histological examination normally formed bone tissue was found, with so-called fibroreticular tissue containing many sinusoidal vessels located between its separate areas.

Electron-autoradiographic investigation in the early stages of formation of the regenerating tissue showed that cells intensively synthesizing DNA and RNA were located exclusively in the vessel walls or in the immediate vicinity of vessels of capillary type (Fig. 1c, d). No cells intensively synthesizing DNA and RNA could be found at a distance from the blood vessels, although many sections were examined. These findings indicate that newly formed vessels of capillary type are an important factor in osteogenesis, for it is only in their walls and in their immediate vicinity that cells synthesizing RNA and DNA, i.e., cells exhibiting high metabolic and proliferative activity, can be found.

It can be tentatively suggested that it is these cells of the vessel wall and its immediate vicinity that are the main source of origin of regenerating bone through the successive transformation of a vascular cell of pericyte type into a fibroblast-like cell, which could be observed in the early stages of formation of the regenerating tissue, and later into an osteoblast. Bone formation can perhaps also take place in other ways, but even so the proliferative activity of the vascular cells must be an important factor in osteogenesis.

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STEREOLOGIC ANALYSIS OF CARDIOMYOCYTE ULTRASTRUCTURAL ORGANIZATION IN RED-CHEEKED SUSLIKS IN DIFFERENT SEASONS

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Hibernation of heterothermic animals is a unique adaptation to unfavorable external environmental conditions [2]. The use of characteristic ecological and physiological features distinguishing hibernating mammals for practical purposes is possible through careful investigation of the organs and systems of these animals and, in particular, of their cardiovascular system. However, the cellular mechanisms lying at the basis of the abrupt seasonal fluctuations in cardiac function in heterothermic animals have by no means been completely elucidated. Among the morphological studies of this problem [6, 7, 10, 13] there have been few attempts to undertake a quantitative study of the tissue components of the myocardium [3]. Quantitative investigations of ultrastructure of the cardiomyocytes of hibernating mammals have yielded contradictory results and have been restricted to a narrow range of morphometric parameters [8, 9, 12].

The aim of the present investigation was a stereologic analysis of the ultrastructural organization of the myocardium of red-cheeked susliks in different seasons of the year.

EXPERIMENTAL METHOD

Altogether 20 hearts of red-cheeked susliks *Citellus erythrogenys* Brandt were studied. These mammals are distinguished by prolonged (7-8 months) hibernation; the body temperature of the active animals is 37°C and of hibernating animals 8°C. Five groups of animals were used: 1) active animals in the fall before hibernation (six susliks), studied at the end of

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