

These experiments thus showed that ethyl alcohol activates the overwhelming majority of ovulating ova in mice and that the parthenogenetic embryos can undergo normal preimplantation development. This simple technique can thus be successfully used to obtain and study induced parthenogenesis in mice.

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#### PARTICIPATION OF BONE MARROW STROMAL PRECURSOR CELLS IN BONE REGENERATION IN TRANSOSSEOUS OSTEOSYNTHESIS

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The authors' observations over many years have shown that prolonged bone formation during distraction osteosynthesis takes place with the active participation of bone marrow. The rapid endosteal reaction is maintained throughout the period of distraction, after which it gradually subsides over a long period, during which time foci of proliferation of osteogenic cells can be observed not only in the "zone of growth," but also throughout the bone marrow of the limb on which the operation has been performed. The intensity of the endosteal reaction depends on various conditions, for example, on the greater or lesser degree of preservation of integrity of the intraosseous vessels and stability of fixation of the bone fragments.

On the basis of the foregoing facts, investigations [1, 4, 7-9] in which high osteogenic activity of stromal precursor cells (SPC) of bone marrow was demonstrated are of considerable interest. The model of distraction osteosynthesis which we have developed, in our opinion affords a basically fresh approach to the study of these cells, the principles governing the function of their reserves, and the elucidation of their role in the regeneration of bone tissue. For this purpose, in the investigation described below, using a technique of monolayer culture, the number and proliferative activity of bone marrow SPC from the long bones were determined during leg lengthening by Ilizarov's method.

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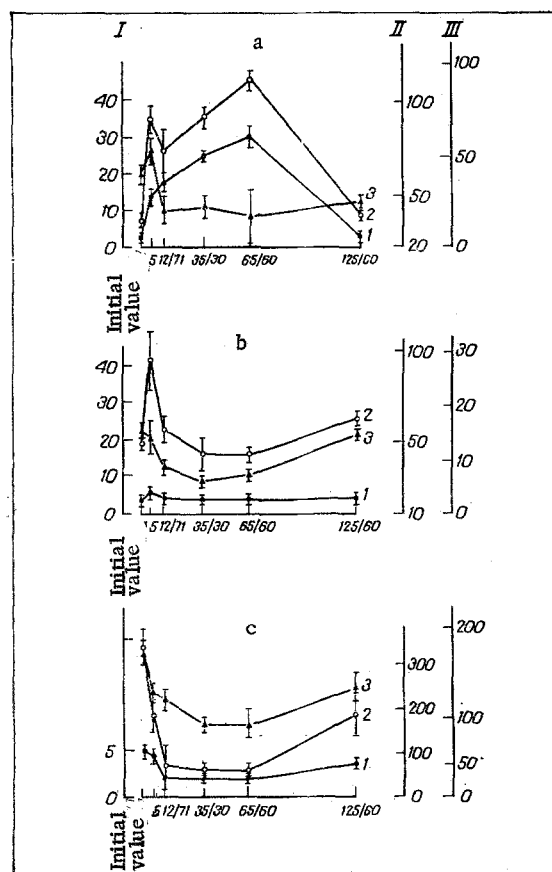


Fig. 1. Dynamics of ECF (1), absolute number of CFU (2) and number of cells in bone marrow (3) of long bones of dogs at stages of leg lengthening by Ilizarov's method: a) tibia undergoing lengthening; b) contra-lateral tibia; c) humerus. Abscissa, time after operation (in days) — 7th, 30th, and 60th days of distraction and 60th day of fixation; ordinate: I) ECF ( $\times 10^5$  bone marrow cells), II) number of cells ( $\times 10^7$ ), III) number of CFU ( $\times 10^3$ ).

#### EXPERIMENTAL METHOD

Altogether 35 mongrel dogs of both sexes, weighing 5–10 kg and aged from 1 to 1.5 years, were used: 30 animals were experimental and 5 were controls. A closed torsion fracture was produced in the experimental animals in the middle third of the right femur by means of Ilizarov's apparatus [3]. Distraction at the rate of 0.25 mm four times a day began five days after the operation. Maximal lengthening amounted to 60% of the initial length of the leg (6–8 cm). After the end of distraction, fixation of the fragments in the apparatus continued for 60 days. The dogs were killed 5, 12, 35, 65, and 125 days after the operation, i.e., before the beginning of distraction, on the 7th, 30th, and 60th days of distraction, and on the 60th day of fixation, respectively. At each time of observation 5 or 6 animals were studied. Under aseptic conditions both tibiae and both humeri were removed from the control and experimental dogs and their marrow was flushed out with medium No. 199. Subsequent treatment of the cell suspension was carried out by the monolayer culture method [6]. The material was seeded in three repetitions in a concentration of  $2 \times 10^6$  bone marrow cells per flask with the addition of 20% homologous inactivated serum from control dogs. The replacement medium contained autologous serum in the same concentration. After incubation for 5–7 days the efficiency of colony formation (ECF) was determined in  $10^5$  explanted bone marrow cells and the absolute number of colony-forming units (CFU) was counted.

The difference hypothesis was tested by the criterion of the median.

## EXPERIMENTAL RESULTS

On the 5th day after the operation (before the beginning of distraction) ECF and a number of CFU were increased in the zone of regeneration. By the 60th day of distraction (Fig. 1a) ECF was 11 times higher than in the control (increased from  $2.8 \pm 0.1$  to  $31.03 \pm 2.7$ ;  $P < 0.001$ ) and the number of CFU was 6 times greater (increased from  $15,233 \pm 599$  to  $92,734 \pm 3561$ ;  $P < 0.05$ ).

In the contralateral tibia ECF also was increased on the 5th day after the operation, but less so than on the side of the operation. Later during lengthening (Fig. 1b), ECF remained higher than initially, with a mean value of  $3.5 \pm 0.1$  ( $P < 0.001$ ), whereas the number of CFU fell, and toward the end of distraction it was  $11,682 \pm 748$  ( $15,233 \pm 599$  in the control,  $P < 0.02$ ). ECF in the humerus at the same time of observation and during the subsequent period of distraction (Fig. 1c) fell compared with the control by 60% (from  $6.08 \pm 0.3$  to  $2.01 \pm 0.6$ ;  $P < 0.001$ ), and the number of CFU fell by 75% (from  $173,504 \pm 24,537$  to  $36,045 \pm 9875$ ;  $P < 0.1$ ).

It was also shown that throughout the period of distraction the number of bone marrow cells in the long bones studied was significantly smaller than in the control (Fig. 1a-c). The number of myelokaryocytes in the lengthened tibia, the contralateral tibia, and the humeri was reduced on average by 26.4, 40.5, and 52.8%, respectively. Negative correlation was found between the values of ECF and the total number of bone marrow cells in the lengthened tibia ( $R = -0.48 \pm 0.14$ ;  $P < 0.05$ ).

By the 60th day of the fixation period the parameters studied had returned close to their initial values.

The results show that the concentration of SPC in the zone of regeneration corresponded to the period of active bone formation, and in our opinion this is evidence that they participate directly in the regeneration process. The decrease in the absolute number of CFU in the control tibia and in both humeri suggests that SPC can migrate into the bone undergoing the lengthening operation, in agreement with suggestions made by other workers [11, 12]. This fact is also evidence that during reparative regeneration of a bone the bone marrow reacts as a single system.

The factors causing the decrease in the number of myelokaryocytes in the marrow of the long bones during distraction are not yet known.

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