NUMBER OF CFU_f PER UNIT VOLUME OF SPONGY BONE IN DIFFERENT PARTS OF THE HUMAN SKELETON

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During cloning of stromal precursor cells of fibroblast colony-forming units (CFU_f) in the bone marrow of laboratory animals, two parameters of their activity are currently used: cloning efficiency calculated per 10^5 explanted nucleated cells, and the number of CFU_f per organ (femur in the rat or ileum in the rabbit) [3, 4].

Cloning efficiency of CFU_f is used to study the CFU_f of human bone marrow as the parameter [2]. This is a relative value, independent of the cell content of the bone marrow and, consequently, it does not characterize the absolute number of CFU_f in the bone marrow of any particular part of the human skeleton. In some forms of pathology accompanied by dysplasia of the bone marrow and bone, however, it is essential to know not only the relative, but also the absolute number of CFU_f in human bone marrow.

The aim of this investigation was to determine the number of CFU_f in 1 cm^3 of human spongy bone.

The investigation was conducted on 155 orthopedic patients with a funnel chest (43 patients aged 4-18 years), with congenital dislocation of the hip (67 patients aged 3-40 years), and with chronic osteomyelitis (45 patients aged 7-14 years).

Spongy bone was taken for investigation into a sterile measuring tube containing medium 199, from places not affected by the inflammatory or degenerative process, during reconstructive-restoration operations.

The volume of spongy bone was measured as the volume of fluid displaced. Bone marrow cells were flushed out of the spongy bone in medium 199 on a magnetic mixer for 30 min. Cloning of CFU_f was carried out by a method developed by the writers and involving the use of rabbit feeder in glass Roux flasks and Petri dishes, using medium 199 with 20% human group AB (IV) serum [1]. Culture was carried out for 12-14 days without change of nutrient medium. The growing cultures were fixed with 96° ethanol and stained by the Romanovsky—Giemsa method. A concentration of cells containing no fewer than 50 fibroblasts was taken as a colony.

The formula for counting the number of CFU_f per unit volume was obtained by logical deductions: the number of CFU_f in 1 cm^3 of spongy bone is equal to the number of colonies of CFU_f grown from the test volume of spongy bone, divided by that volume (V), whereas the CFU_f in the test volume is equal to the number of colonies of CFU_f growing in a flask or Petri dish (K), multiplied by the number of cells flushed out of this volume (N), and divided by the number of transplanted cells (n), for usually not all the cells flushed out are transplanted, but only some of them.

The equation has the form:

$$\text{Number of } CFU_f \text{ in 1 } cm^3 = K.N/V.n.$$

EXPERIMENTAL RESULTS

The results of the study of the number of CFU_f in 1 cm^3 of spongy bone, obtained by the use of this equation, are given in Table 1. Since a definite number of CFU_f in the test volume of spongy bone is directly proportional to the number of cells flushed out, the question naturally arises: were all the nucleated cells flushed out of this volume? During the morphological investigations of the spongy bone after flushing of the cells out of it, it was found that, besides those spaces

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TABLE 1. Number of CFU_f in 1 cm^3 Spongy Bone from Different Parts of the Human Skeleton

Part of skeleton studied	Test object		Number of nucleated cells flushed out of 1 cm^3 of spongy	Cloning efficiency of CFU_f per 10^5 nucleated cells	Number of CFU_f determined in 1 cm^3 of spongy bone, $\times 10^4$	Correction for absolute number of CFU_f in 1 cm^3 of sponge	Absolute number of CFU_f in cm^3 of spongy bone, $\times 10^4$
	Number of fragments of spongy bone	Number of cultures grown					
Sternum	43	173	7,6±0,5	58,07±2,0	3,5±0,09	1,2	4,2±0,01
Ala of ileum	73	242	9,7±0,3	30,16±4,0	1,2±0,07	2,4	2,9±0,16
Proximal metaphysis of femur	39	126	13,06±0,7	10,8±2,0	1,2±0,12	1,07	1,28±0,13

in the spongy bone from which virtually all the bone marrow cells had been flushed out, there were also unopened spaces, completely packed with bone-marrow cells. The cells were most completely flushed out of the spongy bone of the proximal femoral metaphysis, and the largest number of spaces filled with bone marrow remained in the spongy bone of the ala of the ileum. Accordingly, when determining the absolute number of CFU_f per unit volume, a correction has to be introduced for cells which had not been flushed out.

This correction factor can be obtained by comparing the relative proportions of CFU_f and nucleated cells, calculated in accordance with two parameters: cloning efficiency and number of CFU_f in unit volume. These proportions will be equal if all bone marrow cells have been flushed out of the given volume of spongy bone. If, however, the cells had not been completely flushed out, the ratio of the number of CFU_f to the number of nucleated cells, calculated by cloning efficiency, a value independent of the number of flushed-out cells, will be less than the ratio calculated from the number of CFU_f per unit volume, i.e., a value directly proportional to the number of nucleated cells per unit volume, and the same number of times greater as the absolute number of nucleated cells in the given fragment of spongy bone exceeds the number of flushed-out cells.

For the regions of the skeleton studied, we obtained the following ratios of CFU_f to total number of nucleated cells, calculated by cloning efficiency and by number of CFU_f per unit volume: in the sternum 1:1724 and 1:2119, in the ala of the ileum 1:3333 and 1:8281; in the proximal femoral metaphysis 1:10,000 and 1:10,750, respectively.

Comparison of these values yielded conversion factors for determining the absolute number of CFU_f per unit volume and also to calculate the absolute number of CFU_f in the parts of the human skeleton studied. The values are given in Table 1.

Since the investigation was conducted on patients with nonhematologic diseases, and in whom there were no inflammatory or degenerative changes of the spongy bone tested, the values obtained can be regarded as characteristic for these parts of the skeleton in patients of the age groups indicated above.

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