CYTOCHEMICAL CHARACTERISTICS OF CLONES OF HUMAN BONE MARROW STROMAL FIBROBLASTS

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The study of bone marrow stromal fibroblasts by cloning methods *in vitro* has provided the answer to some questions relating to their role in bone tissue formation, the creation of the hematopoietic microenvironment, and regulation of hematopoiesis [4, 5]. Bone marrow fibroblasts and their precursors have so far been studied more in animals than in man. Data on their cytochemical properties have been obtained chiefly by the study of histological bone marrow sections [8, 9]. Some Western workers have studied the cytochemical characteristics of human bone marrow stromal fibroblast cultures [6, 7]. However, the conditions of culture used, permitting growth of primary colonies for 4 weeks, may lead to transformation of the cells [2] and changes in their cytochemical properties.

The object of this investigation was to study cytochemical features of clones of human marrow stromal fibroblasts cultured under conditions permitting good growth until the 10th-12th day.

EXPERIMENTAL METHOD

Bone marrow was obtained from the sternum and iliac crest of six orthopedic patients during reconstructive operations for noncancerous diseases of the locomotor system. Stromal fibroblasts were cloned by the method described previously, using rabbit feeder [1]. A cell suspension was prepared by washing out cells from the spongiosa on a magnetic mixer in medium 199 without the use of any methods of enriching the explanted cell population. The density of explantation did not exceed 5×10^3 nucleated cells per square centimeter of the bottom of the culture vessel. Cultures were fixed in vapor of 10% neutral formalin at the 10th-12th day of culture. Alkaline phosphatase activity in the cells of the colonies was determined by Kaplow's method, acid phosphatase activity after Goldberg and Barka, nonspecific esterase activity by Pearse's method in Löffler's modification, acid nonspecific esterase after Muller et al., and peroxidase after Loele [3].

EXPERIMENTAL RESULTS

During culture under these conditions, discrete clone-colonies of stromal fibroblasts, polymorphic in size and number of cells, grew in the cultures by the 10th-12th day. The number of cells varied from 50 to 500 or more, but large stratified colonies predominated. When cloning efficiency was high (more than 50 per 10^5 explanted cells) confluent growths were formed by that time in the culture flasks.

Data on cloning efficiency of human bone marrow stromal fibroblasts are given in Table 1 and the cytochemical features of the cells of the colonies are indicated.

All colonies of stromal fibroblasts contained cells reacting positively for alkaline phosphatase. The percentage of phosphatase-positive cells in the colonies was fairly constant within the same culture but there were considerable differences between cultures. The end

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TABLE 1. Cytochemical Characteristics of Human Bone Marrow Stromal Fibroblasts

Source of cells	Efficiency of colony formation per 10^5 cells	Alkaline phosphatase, % of cells with positive reaction	Acid phosphatase, % of cells with positive reaction	Nonspecific esterase	Acid nonspecific esterase	Peroxidase
Sternal bone marrow	79±8,0	40,2±0,8	100	—	—	—
	30±1,7	5,2±0,4	100	—	—	—
	40±1,5	29,5±0,6	100	—	—	—
Bone marrow from iliac crest	102±3,9	35,4±3,8	100	—	—	—
	55±0,4	93,8±0,4	100	—	—	—
	94±3,2	8,6±0,7	100	—	—	—

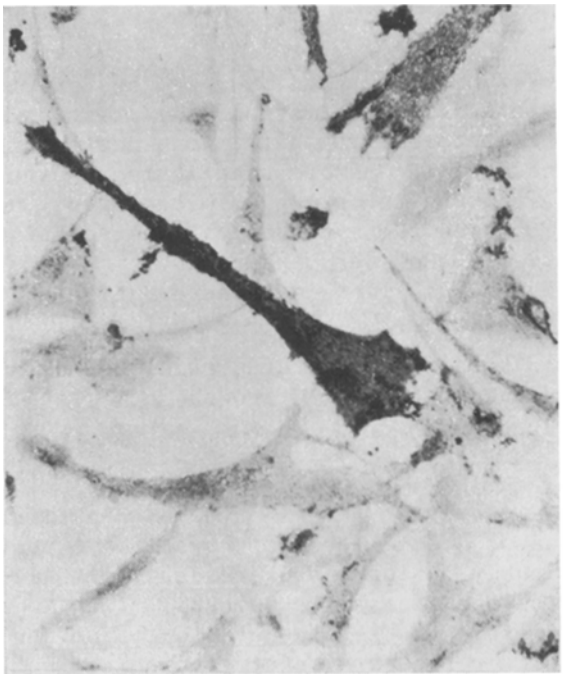

Fig. 1

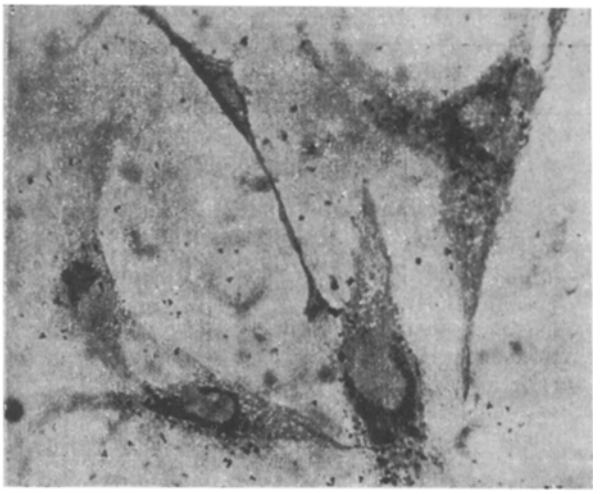

Fig. 2

Fig. 1. Alkaline phosphatase activity in cells of a colony of human bone marrow stromal fibroblasts (400 ×).

Fig. 2. Acid phosphatase activity in human bone marrow stromal fibroblasts (400 ×).

product of the enzyme reaction was uniformly distributed in the form of tiny granules in the cell cytoplasm. Within the same colony, the intensity of the reaction was stronger in some cells than in others (Fig. 1).

Acid phosphatase activity was discovered in all cells of stromal fibroblast colonies without exception. The end product of the reaction, in the form of red granules, was localized in the perinuclear zone of the cells (Fig. 2). Enzyme activity was inhibited on the addition of sodium tartrate to the incubation mixture. The intensity of staining was much lower than in macrophages, which were found occasionally between the colonies.

Unlike data in the literature [6, 7], neutral nonspecific esterase activity was not found in fibroblasts in these experiments. Acid nonspecific esterase and peroxidase activity likewise was not present. The experiments showed that human bone marrow stromal fibroblasts differ in their cytochemical features from histiocyte-macrophages not containing alkaline phosphatase and from cells of the granulocyte series which, besides alkaline phosphatase, also contain peroxidase [3]. This confirms yet again the view that a separate line of precursors exists for stromal fibroblasts, independent of the hematopoietic stem cell. Comparison of the results of this investigation with the cytochemical characteristics of bone marrow reticular cells [8, 9], there are good grounds for considering that the reticular stroma, forming the appropriate framework for hematopoietic cells, is formed from these same colony-forming stromal cells which are the precursors of bone marrow.

Alkaline phosphatase is known to be the marker enzyme of osteoblasts. There is evidence that on retransplantation of colonies of stromal fibroblasts into syngeneic animals bone tissue is formed [5]. We also have observed on more than one occasion deposition of calcium in individual clones in human bone marrow cultures. Consequently, the presence of alkaline phosphatase in cells of the colonies indicates that the precursors forming them are osteogenic precursor cells.

The fact that cells containing alkaline phosphatase and not possessing enzyme activity are present in the same colony indicates that cells at different stages of differentiation are present in the composition of the colonies.

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CIRCADIAN RHYTHM OF SENSITIVITY OF PROLIFERATING LINGUAL AND ESOPHAGEAL EPITHELIAL CELLS TO ADRENALIN

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Adrenalin, the hormone of the adrenal medulla, is known to cause considerable changes in cell proliferation. Most frequently it has an antimitotic action by blocking the end of the G_2 phase of the mitotic cycle [1, 2, 4, 5]. However, there is little information in the literature on the effect of adrenalin on cell proliferation if administered at different times of day or on dependence of the antimitotic effect of the hormone on its dose [3, 5, 6]. The object of this investigation was to study these problems.

EXPERIMENTAL METHOD

Experiments were carried out on 198 noninbred male albino mice weighing 25 g and kept in the animal house with alternation of 12 h daylight and 12 h darkness (daylight from 8 a.m. to 8 p.m.). Some of the mice received a single intraperitoneal injection of adrenalin solution in a dose of 0.5, 1, or 2 μg/g body weight at noon, 4 and 8 p.m., midnight, or 4 or 8 a.m. Control animals were injected with physiological saline at the same times. Experimental animals were killed 40 and 60 min and control animals 50 min after injection of the solutions. The tongue and esophagus were removed from the mice (the esophagus was taken twice — at 8 a.m. and 8 p.m.). After ordinary histological treatment, from 5000 to 25,000 cells in paraffin sections were analyzed in each case and the mitotic index (MI) was calculated in promille. Epithelium from the dorsal surface of the tongue was studied. The numerical results were subjected to statistical analysis by Student's test. Differences were considered to be significant at the $P \leqslant 0.05$ level.

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