medium (Fig. 2). Dependence of [^{3}H]thymidine incorporation on the CM concentration was biphasic in character. In low CM concentrations (10^{-8}-10^{-7} M) an effect of 20-30% was observed, whereas in a concentration of 10^{-6}-10^{-5} M, additional enhancement of proliferation was observed. This may mean that there are two lymphocyte populations with different affinity for CM or that there are two pathways of action of CM on proliferation: one operates in low CM concentrations, the other in high concentrations.

The effect of CM on lymphocyte proliferation thus revealed may be associated with increased membrane permeability for Ca^{++} ions. A similar effect of CM was found on synaptic vesicles in which CM, acting through Ca-dependent protein kinase, stimulates neurotransmitter secretion. More than 10 enzymes which are regulated by CM are known, but all these enzymes are located either in the cytoplasm or on the inside of the plasma membrane. Nevertheless, the possibility cannot be ruled out that CM, on penetrating into lymphocytes, can exert its effect on cell metabolism, thus stimulating proliferation. Further research is required to elucidate the mechanism of action of CM on [^{3}H]thymidine incorporation.

CM is known to be present in all animal tissues. However, it has not been found in blood plasma. It can be tentatively suggested that CM, secreted from foci of injury or inflammation of the tissues, may act as a factor stimulating lymphocyte proliferation in this focus.

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NUMBER OF STROMAL PRECURSOR CELLS IN THE BONE MARROW OF YOUNG AND OLD CBA MICE

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The *in vitro* cloning method enables the number of cells forming fibroblast clone colonies (FCC) in the hematopoietic and lymphoid organs of certain species of mammals to be counted. FCC belong to the category of stromal precursor cells, the progenies of which are responsible for the main functions of the hematopoietic microenvironment [4]. The possible role of the microenvironment of the hematopoietic and lymphoid organs in age changes in hematopoiesis, especially lymphoid, has not been determined. To investigate this problem information is needed on the basic characteristics of the stromal tissue of the hematopoietic organs of old animals.

In the investigation described below the number of FCC was compared in the bone marrow of young and old CBA mice.

EXPERIMENTAL METHOD

The donors of the bone marrow cells were young (1-4 months) and old (24-30 months) female CBA mice. Explantation of the cells into monolayer cultures and subsequent culture were car-

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TABLE 1. EFFC and Number of FCC in Bone Marrow of CBA Mice of Different Ages

Expt. No.	Age of donors of bone marrow, months	EFFC, number of colonies of explanted cells, $\times 10^{-5}$	P	Number of cells in femoral marrow, $\times 10^5$	FCC/organ	P
1	3	0,3±0,03		175	51±4,1	
	12	0,3±0,04		150	40±5	>0,05
	26	0,08±0,004	<0,01	175	13±0,4	< 0.001
2	3	1,7±0,14		170	195±23,7	
	30	2,6±0,10	<0,01	300	840±30	<0,001
3	2	1,0±0,10		133	139±3,8	
	24	1,9±0,14	<0,01	140	266±42,4	<0,05
4	2	1,1±0,10		150	162±17	
	22	2,9±0,20	<0,001	300	872±61	<0,001
	26	2,0±0,05	<0,01	190	397±9	<0,01
5	1	4,5±0,24		80	360±20	
	4	5,7±0,14	<0,001	107	606±15	<0,001
	30	5,2±0,30	<0,05	176	911±52	<0,001
6	4	2,3±0,08		117	274±10	
	27	3,5±0,17	<0,001	187	650±14	<0,001

Legend. FCC/organ signifies number of FCC in marrow of one femur.

TABLE 2. Number of Stromal Fibroblasts in Heterochronous Associations of Bone Marrow Cells from Young and Old Mice

Number of explanted cells ($\times 10^5$) from donors aged		Number of colonies of fibroblasts
2 months	24 months	
40	—	42±0,9
—	40	76±12,2
20	20	92±14,2

ried out by methods described previously [3]. The nutrient medium consisted of 80% medium RPMI 1640 (from Gibco, USA) and 20% calf embryonic serum (N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR). The gaseous phase consisted of a mixture of 10% CO_2 and 90% atmospheric air. Cells suspended in nutrient medium were transferred to plastic flasks with an area of cultural surface of 25 cm^2 (from Corning) or plastic Petri dishes 6 cm in diameter (Falcon Plastics), providing an initial explantation density of 2×10^5 to 3.5×10^5 cells/cm^2 cultural surface of the vessel. To ensure constant efficiency of colony formation, guinea pig bone marrow cells, irradiated in a dose of 45 Gy, were added to the cultures as a natural xenogeneic feeder. On the 12th day the cultures were fixed with 96° ethanol and stained by the Romanovsky–Giemsa method and the number of growing colonies of fibroblasts containing at least 50 cells was counted. On the basis of the results the concentration (efficiency of formation of fibroblast colonies — EFFC) and the total number of FCC in the femoral marrow were determined.

EXPERIMENTAL RESULTS

EFFC in monolayer cultures of CBA mouse bone marrow depended essentially on the type of culture vessel used and the batch of calf embryonic serum. In the initial experiments low efficiency of colony formation was recorded, namely 0.05-0.3 colony per 10^5 explanted cells. In two experiments of the initial series the number of FCC in the bone marrow of the old mice was observed to be lower than in young animals (Table 1, Expt. No. 1). EFFC could be increased considerably if the cells were cultured in plastic flasks (Corning) or plastic Petri dishes 6 cm in diameter (Falcon Plastics) instead of plastic dishes 5 cm in diameter (Millipore), and also after selection of a batch of serum which "supported" growth of colonies of stromal fibroblasts. Under those conditions EFFC reached 1.0×10^{-5} to 5.7×10^{-5} — values characteristic of mouse bone marrow [3]. Meanwhile, EFFC varied significantly from one experiment to another, and for that reason the number of FCC in the bone marrow of young and old mice was compared individually in each experiment.

In all five experiments of this series (Table 1, experiments Nos. 2-6) a significant ($P < 0.05$) increase was observed in EFFC by bone marrow cells of old mice compared with that by bone marrow cells from young donors. The results of experiment No. 5, in which the in-

crease in EFFC in mice aged 30 months compared with the corresponding value for animals aged 1 month was not significant ($P > 0.05$), were an exception.

Comparison of the number of FCC in the marrow of one femur from young and old mice showed a significant (1.5-5 times) increase in the number of stromal precursor cells in the old animals. The differences discovered were perhaps connected with an increase in the total number of nucleated bone marrow cells during aging.

Preliminary experiments showed that constant efficiency of stromal fibroblast colony formation was maintained with optimal initial density of explanted cells. However, a linear relationship between the number of explanted cells and the number of growing fibroblast colonies could not be established in these experiments. Accordingly, the increase in the number of FCC in the bone marrow of the old mice compared with the young donors must be regarded as relative, given an equal initial explantation density.

Age differences in the efficiency of colony formation can hardly be due to a relative deficit of autofeeder cells, maintaining growth of the colonies, in the bone marrow of the young mice and an excess in the old, for addition of xenogeneic feeder cells to the cultures should have removed the hypothetical cause of the differences and led to closer similarity between the values of EFFC for donors of different ages, but this was not observed. The number of growing colonies in heterochronous associations of bone marrow cells (Table 2) exceeded ($P < 0.05$) their number in cultures of cells from young donors, while not differing significantly ($P > 0.05$) from the corresponding figure for bone marrow cell cultures from old mice.

The results are contrary to those of experiments to determine the number of FCC in guinea pig and human bone marrow at different ages [1, 7]. The possible causes of the disagreements are not clear but they may be due to differences between the character of medullary hematopoiesis in donors of different species.

Numerous investigations have shown that the number of hematopoietic stem cells (HSC) in mouse bone marrow remains practically unchanged throughout the life of mammals of this species [8]. An essential role in self-renewal of HSC is played by fibroblasts of the stromal tissue of the microenvironment [2, 4]. In old mice growth of the stroma of a heterotopic bone marrow graft is intensified, and the focus of ectopic hematopoiesis is two to three times larger than that in a young recipient [5]. In long-term bone marrow cultures adherent cells from old mice maintain more intensive and productive self-renewal of HSC than adherent cells from young animals [6]. These data, along with the discovery in the present investigation of an increase in the total pool of stromal precursor cells in the femoral marrow of old mice, can perhaps be regarded as evidence of expansion with age of the stromal basis of the hematopoietic microenvironment. Expansion of this kind — whether topographic or functional — may be the factor which enables the number of HSC to be maintained at a stable level during aging.

Experimental systems of hematopoiesis are the most promising systems for studying the role of connective tissue in age changes in the direction, rate, and regulation of cell differentiation.

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