tion under the influence of the living microflora only. However, the effect on inflammation due to other, nonimmunomodulating mechanisms of action was achieved.

In the more general sense, the difference in morphogenesis of inflammatory changes in the lungs (after injection of a killed and living microflora, after modification by PHA and cyclophosphamide) under conditions leading to similarity between the immune reactions developing in these cases, points to the absence of any close connection between the course of inflammation and the accompanying state of immunity. The absence of any direct connection between inflammation and immunity also was characteristic of other forms of inflammatory processes in the lungs: bronchiectasis and lung abscess [3].

Besides traditional ideas on the direct role of the state of immunity in the genesis of inflammation, the possibility has been demonstrated that changes in inflammatory and immune reactions may be unconnected with each other under the conditions of a pre-existing inflammatory process.

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RESTORATION OF HEMATOPOIESIS IN SUBCUTANEOUS BONE IMPLANTS IN AGING MICE

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KEY WORDS: bone marrow; bone; subcutaneous implantation; aging.

In heterotopic bone marrow transplants and also in the marrow of subcutaneously implanted bone, the hematopoietic cells proper are of recipient origin, whereas the stromal tissue, providing for restoration of hematophoiesis, belongs to the donor [4, 10]. This tissue chimerism enables the use of both experimental models — heterotopic bone marrow transplantation and subcutaneous bone implantation — to assess the microenvironmental functions of the stromal tissue in connection with its repopulation by the recipient's hematopoietic cells [3, 6, 10]. In comparative studies of the stromal tissue of the bone marrow microenvironment during heterotopic transplantation or in subcutaneous implants of the femur in young and old mice, contradictory results have been obtained [1, 9, 10], which can be explained by the particular features of the methods used for transplantation and for assessing restoration of hematopoiesis.

The investigation described below shows that removal of the contents of the medullary cavity before subcutaneous implantation of bone may reveal age differences in the ability of the stromal tissue of bone marrow to maintain restoration of hematopoiesis in the implanted bone.

EXPERIMENTAL METHOD

In experiments on recipient CBA mice aged 2-3 months, anesthetized with Ketalar (ketamine), one femur each from a young (2-3 months) and old (24-26 months) syngeneic donor was implanted subcutaneously on the lateral surface of the chest on each side. The contents of the medullary cavity of some bones were removed before implantation, and the bone marrow was destroyed with an injection needle, and repeatedly flushed out with cold McCoy's 5A nutrient

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TABLE 1. Colonization of Implant with Hematopoietic Cells (M ± m)

Parameter	Donors of implants			
	young		old	
	1	2	1	2
Number ($\times 10^5$) of nucleated cells	27.58±2.93 (23)	29.75±3.66 (22)	28.54±3.65 (26)	11.23±3.05 (18)
Number of CFU-GM per 10^{-5} nucleated cells	25.85±4.31 (22)	26.51±5.20 (21)	18.46±3.75 (24)	5.08±2.44 (12)
Total number of CFU-GM	712.97±108.55 (22)	794.90±174.00 (21)	715.10±192.30 (24)	95.00±55.00 (12)

Legend. 1) Implants grafted without removal of contents of medullary cavity; 2) implants grafted after removal of contents of medullary cavity. Numbers in parentheses show number of implants studied.

medium (from Serva, West Germany). The microenvironmental function of the stromal tissue of the bone marrow regenerating in the implant was assessed 2-2.5 months after the operation by determining colonization of the medullary cavity with nucleated hematopoietic cells and with precursor cells of granulocytic-macrophagal (CFU-GM) colonies in semisolid agar cultures [7].

EXPERIMENTAL RESULTS

Implants from young and old donors 2-2.5 months after the operation did not differ significantly from one another ($p > 0.1$) as regards either the number of nucleated cells or the number of CFU-GM (Table 1). Meanwhile repeated observations, including our own experiments on CBA mice [2] are evidence that the number of cells in the bone marrow of mice aged about 2 years is approximately twice their number in the bone marrow of young animals (2-4 months). Hence it follows that restoration of the number of bone marrow cells in femoral implants from old donors, according to the results of our experiments, takes place twice as slowly as in implants from young donors. However, we know that the internal milieu of the recipient [6] has a marked influence on the size and functional properties of the stromal tissue of bone marrow regenerating in heterotopic grafts, and that this influence may be age-specific [1]. It is thus impossible to identify or rule out any possible differences in the regenerative potentials of the bone marrow stroma depending on age, by the use of the routine method of subcutaneous implantation of the femur. Differences of this kind were found after exposure of the stromal tissue to an additional procedure — by mechanical destruction of the tissue before implantation of the bone. Preliminary removal of the contents of the femoral medullary cavity before transplantation was found to have no significnat effect on restoration of hematopoiesis in implants from young donors (Table 1). Meanwhile, in implants transplanted from old donors after removal of the bone marrow, the number of hematopoietic cells showed a distinct fall — both compared with "intact" implants from animals of the same age ($p < 0.01$) and with implants from young donors ($p < 0.001$), including implants with clearance of the medullary cavity before transplantation ($p < 0.001$; Table 1).

The results are thus evidence that mechanical removal of the greater part of the marrow from the femur before its transplantation sharply inhibits regeneration of hematopoiesis in an implant from an old, but not from a young donor.

It was shown previously [10] that tissue of regenerating bone marrow in subcutaneous implants of the femur in mice is of donor origin irrespective of whether the bone is transplanted with or without the bone marrow. In the latter case regeneration of the stromal microenvironment evidently takes place on account of the subendosteal layer of precursors remaining after evacuation of the contents of the medullary cavity. It is natural to suggest that in old mice the number of subendosteal stromal precursors and (or) their regenerative potential will be less than in young mice, and this can be attributed to the effects of age. In a recent experimental investigation [9] no radial gradient of the concentration of cells formed in monolayer culture of a colony of stromal fibroblasts could be found in the bone marrow of the long bones in young mice. Such a gradient can be established in old age, for example, as a result of progressive osteoporosis [6], and is exhibited as a decrease in the number of stromal precursors in the subendosteal layer of the bone marrow or prevalence of precursors with reduced regenerative potential in that zone.

The results of the present investigation are evidence that the stromal tissue of the bone marrow microenvironment in mice undergoes significant structural changes with age. However, these changes can be revealed only under experimental conditions that simulate an extraordinary local regenerative demand.

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INHIBITION OF α-FETOPROTEIN SYNTHESIS IN ADULT MOUSE HEPATOCYTES BY DEXTRAN SULFATE IN VIVO AND IN VITRO

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Much evidence has recently been published that charged polymers are activators of cells of the immune system [4, 5]. The mechanism of action of polyelectrolytes on cells has not been finally elucidated, but even at this stage it can be asserted that it is largely connected with direct interaction of charged macromolecules with components of the plasma membrane [4, 5]. The use of polyelectrolytes as agents regulating various cell functions through the membrane may also be interesting when other cell types are to be acted upon.

The effect of polyelectrolytes on primary monolayer cultures of adult mouse hepatocytes was investigated. It was found previously that hepatocytes from the intact adult mouse liver in culture change their functional activity significantly and begin to synthesize the embryospecific protein α-fetoprotein (AFP) intensively [2, 3, 8]. Despite numerous attempts, it has not yet proved possible to regulate this process. One synthetic polyanion, dextran sulfate (DS), has been found to induce considerable morphological changes in hepatocytes in culture, when AFP synthesis is appreciably inhibited; DS also inhibits AFP synthesis in vivo in the mouse liver regenerating after CCl_4 poisoning.

EXPERIMENTAL METHOD

A suspension of hepatocytes was obtained from the liver of adult C57BL/6 mice after perfusion with solutions of EGTA and collagenase. The procedure used to isolate and culture the cells was fully described previously [2, 3]. On the 3rd-6th day of culture the hepatocytes

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