In previous investigations the writers obtained data indicating that transfer of the bone marrow microenvironment takes place as a result of survival of stromal mechanocytes with osteogenic potential, and not macrophages [3]. It has so far proved impossible to grow mouse bone marrow fibroblasts in culture without admixture of macrophages. There is reason to suppose that in cultures of mouse bone marrow cells the microenvironment is transferred by adhesive osteogenic fibroblasts, which have multiplied in culture. The use of sponges for transplanting cells grown in culture enabled successful transfer of the microenvironment to take place by stromal cells from primary mouse cultures, which has hitherto been impossible. In the present investigation transfer took place more effectively and constantly than by transplantation of strains of rabbit bone marrow fibroblasts (when sponges were not used). It must also be pointed out that adhesive cells from bone marrow cultures used to transfer the bone marrow microenvironment during transplantation were from a line of mice (CBA) whose adhesive cells are known not to maintain its hematopoietic microenvironment in culture [7]. This may indicate a difference between the cells responsible for each of these two processes. It is interesting to note that, as the large number of osteogenic cells revealed, more of them accumulate in the layer of adhesive cells of Dexter cultures, evidently, than in bone marrow used for explantation.

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FORMATION OF FOCI OF MYELOID CELLS ON COLONIES OF THYMUS AND BONE MARROW FIBROBLASTS IN MONOLAYER CULTURE

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KEY WORDS: colonies of stromal thymus and bone marrow mechanocytes; foci of myeloid cells.

Stromal mechanocytes (fibroblasts) of hematopoietic and lymphoid organs play the leading role in the local regulation of hematopoiesis [3]. It has recently been shown that stromal mechanocytes of different hematopoietic organs bind differentiated granuloid cells in vitro to an equal degree [2].

In the present investigation the ability of stromal fibroblasts of bone marrow and thymus to interact in culture with hematopoietic precursor cells forming colonies of myeloid cells was studied.

EXPERIMENTAL METHOD

Noninbred guinea pigs weighing 180-250 g and (CBA × C57BL)F_1 mice weighing 18-22 g were killed with chloroform. Filtered suspensions of mouse and guinea pig femoral marrow cells and mouse thymus cells were prepared by the standard method [6] in Fisher's medium. Between 1×10^6 and 2×10^6 bone marrow cells or 6×10^6 and 12×10^6 mouse thymus cells were explanted into plastic flasks and Petri dishes (the area of the

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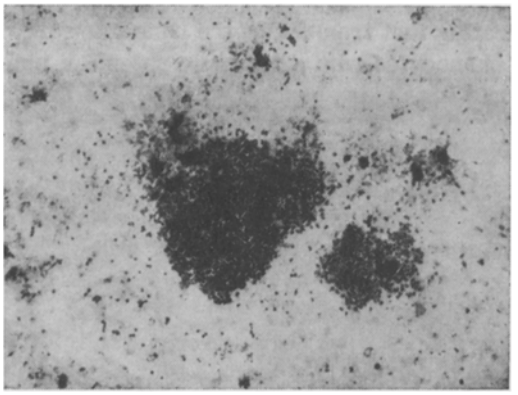

Fig. 1

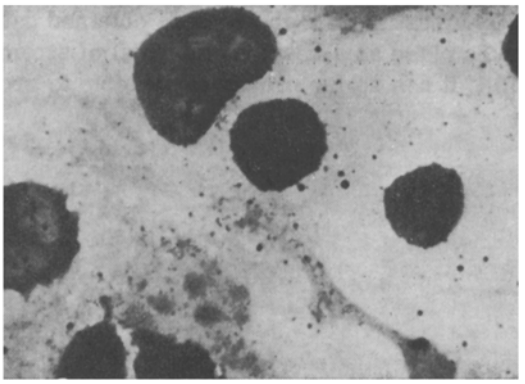

Fig. 2

Fig. 1. Foci of myeloid cells on colonies of thymus fibroblasts, on 4th day after secondary addition of bone marrow cells. Stained with Sudan black and methylene blue, objective 4, ocular 10.

Fig. 2. Region of myeloid focus in the same culture. Myeloid cells of different degrees of maturity lie directly on the surface of a fibroblast. Stained with Sudan black and methylene blue, objective 100, ocular 10.

TABLE 1. Formation of Myeloid Foci from Colonies of Bone Marrow and Thymus Fibroblasts ($M \pm m$)

Origin of fibroblasts	Time after transplantation of bone marrow cells, days	Number of cultures	Mean percentage of fibroblast colonies occupied by myeloid cells	Mean number of myeloid foci per occupied fibroblast colony	Mean size of myeloid focus, number of cells	Mean number of myeloid cells per occupied fibroblast colony
Bone marrow	2—4	8	15,2±3,3	2,1±0,4	42,2±4,6	90,6±10,0
	5—7	22	13,7±1,9	1,6±0,1	87,0±19,9	134,2±19,5
Thymus	2—4	7	35,4±5,1	2,9±0,4	86,6±5,2	246,8±27,2
	5—7	7	41,4±4,0	3,0±0,3	329,4±37,3	983,7±140,1

bottom was taken to be 25 cm^2) together with 7×10^6 to 10×10^6 guinea pig bone marrow cells irradiated in a dose of 4000 rads, to act as the feeder. The culture medium consisted of 80% Fisher's medium and 20% embryonic calf serum; for each 100 ml of medium 0.5 ml of 1 M HEPES buffer solution, 60 mg L-glutamine, and 6000 units each of penicillin and streptomycin also were added. Cultivation took place at 37°C in an atmosphere consisting of a mixture of air with 5% CO_2, saturated with water vapor. Half the medium was changed after 7 days of culture. On the 11th-14th day the medium with the floating cells was removed and a freshly prepared suspension of syngeneic mouse bone marrow cells added to the primary cultures in a dose of 1×10^5 to 2×10^5 cells in RPMI-1640 medium with 20% embryonic calf serum and with the additives mentioned above. Cultivation continued for a further 2-7 days, after which the cultures were washed with medium No. 199, fixed with calcium—formol, and stained with Sudan black [7] and counterstained with methylene blue. Some of the cultures 3 days after secondary addition of bone marrow cells were treated with [^{3}H]thymidine (specific activity 22.6 Ci/mmole) in a concentration of 0.2 μCi/ml. After 24 h these cultures were washed with medium No. 199 and fixed with ethyl alcohol. They were then treated with 3% perchloric acid for 20 min, coated with type M emulsion, and developed for 22 days.

EXPERIMENTAL RESULTS

The 11-14-day primary monolayer cultures of mouse bone marrow and thymus cells consisted of numerous discrete colonies of fibroblasts and a certain number of macrophages [6]. Sudan-positive cells were not present in these cultures. Conversely, in cultures fixed 2-7 days after secondary transplantation of bone marrow cells discrete foci of myeloid cells, giving a strongly positive reaction to staining with Sudan black, were distributed. The effectiveness of formation of these foci in cultures of bone marrow fibroblasts was 5-28 and in cultures of thymus fibroblasts 27-82 per 10^5 added bone marrow cells. The myeloid foci were present only on the surface of the fibroblast colonies (Fig. 1), and were never found on the surface of the plastic or on macrophages. Most foci consisted of myeloid cells at different stages of maturity. Besides small, strongly Sudan-positive stab cells and polymorphonuclear granulocytes, larger Sudan-positive forms

TABLE 2. Dependence of Formation of Myeloid Foci on Size of Fibroblast Colonies (data for cultures fixed 5-7 days after retransplantation of bone marrow cells)

Index	Colonies of thymus fibroblasts			Colonies of bone marrow fibroblasts		
	large	medium-sized	small	large	medium-sized	small
Distribution among all fibroblast colonies, %	16,8	22,3	60,9	15,3	37,4	47,3
% of colonies occupied by myeloid cells	94,2	58,5	23,6	45,0	10,9	4,3
Mean size of myeloid focus (number of cells)	433,5	198,5	158,8	131,2	92,2	66,3
Mean number of myeloid cells per colony of fibroblasts ($M \pm m$)*	2164,7±243,2	479,6±38,4	291,1±18,3	272,0±40,6	115,2±18,0	66,3±13,4

*For each of the three groups of colonies differences between colonies of bone marrow fibroblasts and thymus fibroblasts are statistically significant ($P < 0.001$).

also were found, with a ring-shaped nucleus – myelocytes and metamyelocytes (Fig. 2), and also (but much less frequently) Sudan-negative promyelocytes or myeloblasts. The proportion of mature cells increased with an increase in the duration of culture. The size of the foci also increased in the course of culture. The most numerous foci in colonies of bone marrow fibroblasts 2-4 days after secondary transplantation were those containing 20-40 myeloid cells, but after 5-7 days the most numerous colonies contained 50-100 cells; the corresponding numbers for colonies of thymus fibroblasts were 50-150 and 200-500 cells respectively. Some foci attained a larger size (up to 550 cells in bone marrow and up to 6400 cells in thymus cultures). In both thymus and bone marrow cultures from 88 to 100% of cells of myeloid foci incorporated [^{3}H]thymidine.

The fraction of fibroblast colonies occupied by myeloid cells was independent of the duration of culture (Table 1), but at the same time the fraction of occupied colonies was greater in thymus fibroblast cultures than in bone marrow cultures ($P < 0.01$). The mean size of the myeloid focus and the mean number of myeloid cells per occupied fibroblast colony increased significantly during culture; in both early and late cultures both these values were significantly higher for thymus fibroblasts than for bone marrow fibroblasts ($P < 0.001$).

Fibroblast colonies are divided into three groups in Table 2: large (over 5 mm in diameter), medium-sized (2.5-5 mm), and small (under 2.5 mm). It will be clear from Table 2 that with a decrease in size, the fraction of colonies occupied by myeloid cells, the mean size of the myeloid focus, and the mean number of myeloid cells per colony all decreased. However, all these indices in each group were significantly higher for colonies of thymus fibroblasts than bone marrow. These observations show that differences in the formation of myeloid foci on colonies of thymus and bone marrow fibroblasts do not depend on differences in the size of these colonies.

The dynamics of growth of the foci and autoradiographic data show that cells forming myeloid foci are in a state of intensive proliferation. This fact, and also the discrete character of the foci, suggest that myeloid foci arise from a limited number of hematopoietic precursor cells present in the suspension of bone marrow cells added secondarily. Which cells in fact give rise to the foci is not clear. However, since many foci contain large numbers of myeloid cells (from several hundreds to several thousands), it can be tentatively suggested that early, morphologically unidentifiable hematopoietic precursor cells are concerned.

Colonies of fibroblasts which developed in cultures in the cell densities used for primary explantation are clones of stromal mechanocytes [1, 3]. Colonies of stromal thymus fibroblasts stimulate the development of myeloid foci much better than colonies of stromal bone marrow fibroblasts. Since myeloid foci develop directly on the surface of the fibroblasts, this difference may perhaps be due to differences in the intercellular contacts between hematopoietic and stromal cells. Thymus fibroblasts evidently bind or induce proliferation and differentiation of a greater number of hematopoietic precursor cells than bone marrow fibroblasts, and they also communicate a greater proliferative stimulus to the precursor cells. This result can also be explained by the different ability of stromal cells of bone marrow and thymus origin to secrete substances with colony-stimulating activity [5], although the question of the necessity of a colony-stimulating factor for the maintenance of myeloid hematopoiesis in cultures on a substrate of stromal cells still remains highly contradictory [4, 8].

On the whole, by contrast with the morphologically different granuloid cells which attach themselves equally to stromal mechanocytes of different hematopoietic organs [2], precursor cells forming myeloid foci interact unequally with stromal mechanocytes of bone marrow and thymus origin.

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EMBRYOTOXIC ACTION OF CYCLOPHOSPHAMIDE AFTER BIOTRANSFORMATION IN CULTURES OF POSTIMPLANTATION RAT EMBRYOS

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The rapidly increasing number of pharmacological agents and also of chemical compounds polluting the environment makes the development of highly sensitive test systems for the detection of potential teratogens a particularly urgent task [1]. The use of cultures of postimplantation embryos of laboratory rodents for these purposes is spreading in the practice of research workers studying problems in applied teratology [4, 8]. However, the method of testing teratogens used in such cases, by direct addition of the test substances to the culture media [4, 6, 8, 11], can be used to detect the embryotoxic properties of the substances themselves, but not of their possible metabolic products.

Many workers are currently engaged on a search for approaches to the study of the embryotoxicity of metabolic conversion products of chemical substances formed directly in culture media to which either components of liver enzyme systems [7, 10] or cells of mammalian embryos [10] are added.

The object of this investigation was to study the possibility of using the microsomal fraction (MF) of rat liver and the essential cofactor NADPH [5] to bring about biotransformation of the known antitumor agent cyclophosphamide (CP) to teratogenic products in cultures of postimplantation rat embryos and to compare the sensitivity of this test system with that of the generally adopted method of studying embryotoxicity in pregnant animals [3].

EXPERIMENTAL METHOD

Experiments were carried out on embryos of 50 noninbred albino rats. On the 10th day of pregnancy the animals were killed by cervical dislocation and the uterus with embryos removed under sterile conditions. Embryos at the stage of formation of the 1st-3rd pairs of somites were cultured by New's method [12] at 37°C for 48 h in revolving (30 rpm) 100-cm^3 glass tubes containing 7.5 ml of homologous blood serum. For biotransformation of CP in vitro not more than 0.5 ml (total volume) of the necessary components were added to the incubation medium, so that their final concentrations in the medium, depending on the character of the experiment, were: protein of MF 0.4-2.0 mg/ml, NADPH 1 mM, CP 0.001-500 μg/ml. At the end of incubation

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