moreover, include not only highly differentiated, but also stem cells [2]. Stem cells spend a large part of their life cycle in a resting state. Electron-microscopic investigations have shown that nucleoli of differentiated cells are characterized by certain definite morphological features (circular nucleoli). On transition of the cells from the resting state into the cell cycle and, in particular, during PHA-dependent stimulation of proliferation of human peripheral blood leukocytes, the fine structure of the nucleoli undergoes certain changes [15]; the order of these changes, moreover, is opposite to that observable during erythroid cell differentiation [13]. It is striking that the nucleolus, as a morphological structure, undergoes specific changes first in all these cases [12]. When the data given in Table 1 are analyzed, a similar relationship can be observed.

The data described above suggest that activation of the nucleoli and an increase in their number cannot be specific characteristics of any particular pathological state. Any disease connected with disturbance of the "proliferative homeostasis" of a tissue will lead to a change in the ratio between proliferating and quiescent cells and to an increase in the number of nucleoli.

LITERATURE CITED

1. R. Adams, Methods of Cell Culture for Biochemists [in Russian], Moscow (1983).
2. O. I. Epifanova, V. V. Terskikh, and V. A. Polunovskii, Resting Cells [in Russian], Moscow (1983).
3. A. I. Kononskii, Histochemistry [in Russian], Kiev (1976).
4. P. F. Rokitskii, Biological Statistics [in Russian], Minsk (1967).
5. G. I. Roskin, Microscopic Techniques [in Russian], Moscow (1946).
6. S. M. Terekhov, Tsitologiya, 23, 717 (1981).
7. J. Karasek, J. Janula, and K. Smetana, J. Invest. Derm., 77, 369 (1981).
8. M. S. Kilyovska and L. I. Novoselska, Acta Med. Bulg., 9, 10 (1982).
9. V. I. Kukharenko, K. N. Grinberg, and A. M. Kuliev, Hum. Genet., 42, 157 (1978).
10. G. Mihaescu and L. Gavrila, Rev. Roum. Biol. Anim., 26, 127 (1981).
11. D. Salk et al., Cytogenetics, 30, 108, 1981).
12. H. G. Schwarzacher and F. Wachtler, Hum. Genet., 63, 89 (1983).
13. K. Smetana and Z. Likovsky, Z. Zellforsch., 133, 367 (1972).
14. H. Sugimori et al., Acta Cytol., 26, 439 (1982).
15. F. Wachtler, A. Ellinger, and H. G. Schwarzacher, Cell Tissue Res., 213, 351 (1980).

PRECURSORS OF TRANSIENT SPLENIC COLONIES IN THE MOUSE EMBRYONIC LIVER

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Colonies in the spleen of irradiated mice are known to be produced by polypotent, highly self-supporting hematopoietic precursor cells [5]. In this connection the identity of hematopoietic stem cells (HSC) and of cells forming colonies in the spleen (CFU_S) appeared evident. The two terms are often used as synonyms. Nevertheless, no strict proof of this dogma has yet been obtained; indeed, on the contrary, there is weighty, although indirect, evidence of differences between HSC and CFU_S [1]. It has recently been shown experimentally that some splenic colonies are produced by bone marrow cells incapable of self support [2]. These colonies are transient in nature, i. e., they can be detected 7–9 days, but not 10–12 days after injection of the cells. Transient colonies also are distinguished by the fact that they contain no polypotent precursors capable of forming mixed colonies of erythroid and myeloid cells in culture (CFU_{em}), and also unipotent precursors of granulocytes and macrophages (CFH_c) and of erythrocytes (PFU_e) although more mature erythroid precursors (CFU_e)

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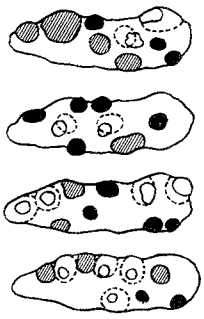

Fig. 1. Scheme of mapping splenic colonies 7 and 11 days after injection of embryonic liver cells. Black areas – colonies observed only after 7 days; obliquely shaded areas – colonies observed only after 11 days. Continuous and broken lines surround colonies observed after 7 and after 11 days respectively.

TABLE 1. Macroscopic Colonies on Outer Surface of Spleen 7 and 11 Days after Injection of Embryonic Liver Cells

Age of donors of embryonic liver, days	Number of colonies mapped		Number of colonies which		
	after 7 days	after 11 days	remained	disappeared	appeared de novo
12—13	103	74	51	52	23
17	48	48	23	25	25
18	22	19	13	9	6

are found in them. Hence it can be concluded that transient colonies are produced not by stem cells, but by their more mature progenies (these will subsequently be called CFU_{tr}), possibly identical with early erythroid precursors.

These facts are of fundamental importance for the description of HSC, for in the overwhelming majority of investigations, colonies in the spleen were recorded 7–9 days after injection of the cells and, consequently, not only HSC, but also CFU_{tr}, were included in the counting. CFU_{tr} have recently been found in adult mouse bone marrow. It is no less important to establish whether CFU_{tr} are an essential step in differentiation of all HSC, and in particular, of embryonic HSC. The investigation described below was devoted to the study of this problem.

EXPERIMENTAL METHOD

Experiments were carried out on female (CBA × C57BL)F_1 mice aged 8–12 weeks and also on embryos of this same genotype obtained from females at the 12th–13th and 17th–18th days of pregnancy. Recipient mice were irradiated with ^{137}Cs γ-rays on the IPK apparatus in a dose of 13 Gy and with a dose rate of 0.25 Gy/min. The embryonic liver was minced in a glass homogenizer and $1 \cdot 10^5$ to $1.6 \cdot 10^5$ cells were injected intravenously into recipients in the course of 2 h after irradiation. After 7 days, under general anesthesia, laparotomy was performed on some of the recipients, and the distribution of colonies on the outer surface of the spleen was recorded with a binocular loupe. To facilitate mapping of the colonies, an ocular with grid was used. After 11 days the mice were killed and the colonies again mapped both on fresh spleens and on spleens fixed in Bouin's fluid. The number of CFU_{em}, PFU_e, and CFU_c in individual 7- and 11-day splenic colonies was studied in some of the recipients. For this purpose cells of each individual colony were explanted into two wells of a 24-well plate into 0.3 % agarized medium (based on medium DMEM with serum of mice treated with endotoxin as stim-

TABLE 2. Precursors Forming Colonies in Vitro in Individual 7- and 11-Day Splenic Colonies, Produced by Embryonic Liver Cells

Age of donors of embryonic liver, days	7-Day splenic colonies				11-Day splenic colonies			
	agarized cultures		methylcellulose cultures		agarized cultures		methylcellulose cultures	
	number of colonies studied	number of colonies without precursors	number of colonies studied	number of colonies without precursors	number of colonies studied	number of colonies without precursors	number of colonies studied	number of colonies without precursors
12—13	24	12 (50%)	24	11 (45%)	—	—	36	6 (17%)
18	—	—	24	12 (50%)	19	6 (31%)	22	2 (9%)

ulator of CFU_c), and into two wells into 0.8% methylcellulose medium (based on medium DMEM) to which medium conditioned by C57BL mouse spleen cells, stimulated by pokeweed lectin [3] was added as stimulator of CFU_{em}, PFU_e, and CFU_c, and serum of irradiated mice treated with phenylhydrazine [4] was added as a source of erythropoietin. The cultures were incubated at close to 100% humidity at 37°C in an atmosphere with 5% CO_2 for 7—8 sec (agarized cultures) or 10—11 sec (methylcellulose cultures), after which the number of cell aggregates was counted under an inverted microscope.

EXPERIMENTAL RESULTS

Colonies in the spleen of 21 recipients were mapped. Typical schemes of the mapped spleens are shown in Fig. 1. Clearly only some of the 7-day colonies survived until the 11th day and many of them disappeared. Among the 11-day colonies there were some which had appeared de novo and were not present on recording after 7 days. The complete data are shown in Table 1. After addition of both early and late embryonic hematopoietic cells a large proportion (40—50%) of the 7-day splenic colonies consisted of transient colonies. Meanwhile about one-third of the 11-day colonies consisted of colonies not recorded after 7 days, from which it follows that not all CFU_s give macroscopic colonies by the 7th day after injection. The reason for this may be either the delayed onset of proliferation of such CFU_s after transplantation or the long generation time of their progenies.

Not all colonies in the spleen contained precursors forming colonies in culture (Table 2). About half of the 7-day colonies (45-50%) did not contain early precursors capable of forming colonies in culture. This corresponded completely to the fraction of transient colonies detected by the mapping method. Meanwhile most of the 11-day colonies (80—90%) contained early hematopoietic precursor cells, including CFU_{em}, which were found in about one-fifth of the 11-day colonies.

On the whole these results are very similar to those obtained in experiments with adult mouse bone marrow [2]. In the embryonic liver CFU_{tr} account for up to 50% of the total cell population capable of forming macroscopic colonies in the spleen of irradiated recipients. It can be tentatively suggested that CFU_{tr} are an essential step in differentiation of, evidently, all categories of HSC. Whatever the case they are found not only in adult mice, but also during embryogenesis — in the earliest periods of hepatic hematopoiesis.

LITERATURE CITED

1. I. L. Chertkov and A. Ya. Fridenshtein, Cellular Basis of Hematopoiesis [in Russian], Moscow (1977).
2. M. C. Magli, N. N. Iscove, and N. Odartchenko, Nature, 295, 527 (1982).
3. D. Metcalf, J. R. Johnson, and T. E. Mandel, J. Cell. Physiol., 98, 401 (1979).
4. P. E. Tambourin, F. Wendling, D. Gallien-Lartigue, et al., Biomedicine, 19, 112 (1973).
5. J. E. Till and E. A. McCulloch, Radiat. Res., 14, 213 (1961).