HEMATOPOIESIS IN HUMAN EMBRYONIC LIVER ORGAN CULTURE

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It was shown by the method of multiple organ cultures on Millipore filters that hematopoiesis of predominantly erythroid type is maintained for a long time (over 1.5 months) in cultures of human embryonic liver. The general morphology of 7-50-day-old cultures was studied and described. The myeloid population of cells (the number of colony-forming units) was virtually exhausted by the 14th-16th day of culture.

KEY WORDS: *human embryonic liver; hematopoiesis in culture; colony-forming units.*

Myeloid hematopoiesis is maintained in explants of mouse embryonic liver in organ culture. Proliferation and differentiation of hematopoietic cells in such cultures are observed for a long time, and hematopoietic stem cells, capable of forming colonies (erythroid, myeloid, megakaryocytic, and mixed) in the spleen of irradiated mice persist throughout the period in culture [3].

Information on prolonged maintenance of hematopoiesis in human embryonic liver *in vitro* is extremely limited. One investigation of this type was that undertaken by Benevolenskaya [1], who observed hematopoiesis for a relatively long time (7-10 days) in explants of human embryonic liver during culture in a plasma clot. During culture of human embryonic liver in agar, death of the hematopoietic cells passed as early as on the fourth day [6].

The investigation described below demonstrated that human embryonic liver can be maintained in culture for a long period (up to 50 days) by the organ culture method and that the kinetics of precursor cells of the granulocytic series can be studied.

EXPERIMENTAL METHOD

Experiments were carried out on 7-14-week-old human embryos. Intact embryos, kept in medium No. 199 with the addition of antibiotics, were used. Explantation of the liver was carried out under sterile conditions 12-24 h after removal of the embryos at therapeutic abortion. The embryos were washed twice in medium No. 199, then placed in complete nutrient medium, the liver was removed and cut into fragments, and these were grown by the multiple organ culture method [3, 4]. The number of precursor cells of the granulocytic series (CFU_C) at different times of cultivation was determined by the cloning in agar method [2, 5]. Human peripheral blood leukocytes were used as the stimulating factor [5]. The general morphology of the human embryonic liver cultures was studied in total preparations stained with hematoxylin and mounted in balsam and also in films stained with azure–eosin.

EXPERIMENTAL RESULTS

During the first 7-10 days in culture, a large zone of necrosis with pycnotic nuclei and residues of hemosiderin, located in the center of the explant, could be seen in the total

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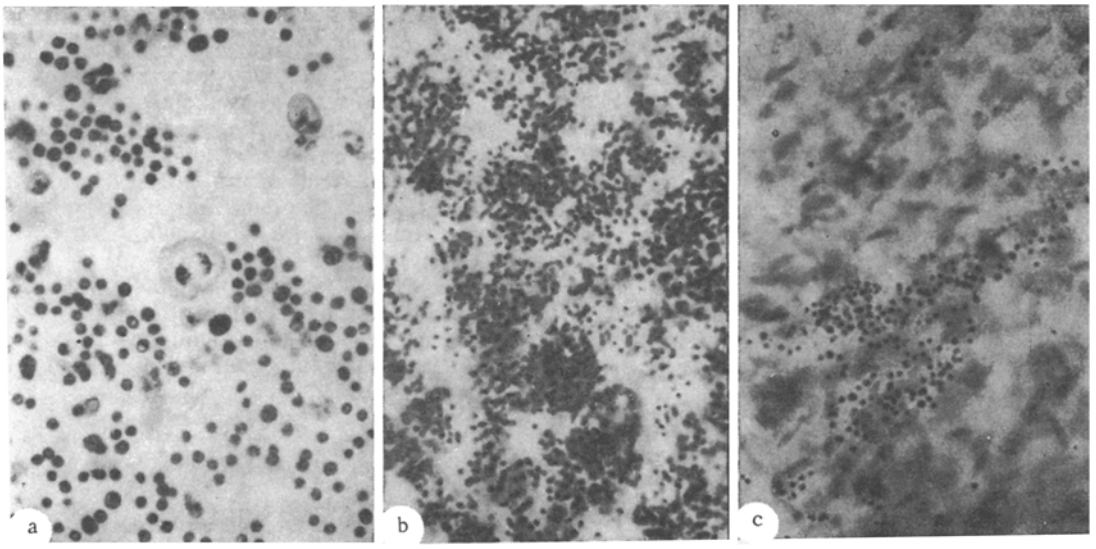

Fig. 1. Photomicrographs of human embryonic liver cultures (total preparations, 20×): a) 7-day-old culture: mitotic figures, cells mainly of erythroid series, granulocytic cells form small groups; b) 30-day-old culture: erythroid cells from extensive fields; c) 50-day-old culture: many macrophages act with hemosiderin, erythroid cells occur as small groups.

TABLE 1. Cells Composing Explants at Different Stages of Cultivation (in %)

Cultivation time, days	Myeloblasts	Promyelocytes	Myelocytes	Metamyelocytes	Lymphocytes	Promegaloblasts	Basophilic megaloblasts	Polychromatophilic megaloblasts	Polychromatophilic megaloblasts
0	—	0,5	—	—	—	5,0	16,0	44,0	34,0
5	—	—	2,0	—	3,0	—	6,5	19,0	69,5
7	1,0	1,5	—	—	0,5	—	4,0	43,0	50,0
11	5,5	3,0	2,5	5,0	7,5	0,5	2,5	31,0	42,5
50	—	—	2,0	—	2,0	6,0	6,0	38,0	46,0

TABLE 2. Number of Living Cells and CFU_c in Human Embryonic Liver Cultures

Cultivation time, days	Number of experiments	Number of living cells per explant (× 10^5)	Number of CFU_c	
			per 10^5 cells	per explant
0	3	13,16±3,3	66,87±6,9	887,0
1	3	7,89±1,25	24,3±3,8	182,1
5	4	8,51±1,2	70,5±7,2	636,6
7	6	6,48±1,2	26,8±13,6	131,5
10	5	3,02±0,85	22,6±6,3	60,9
14	5	1,45±0,32	22,0±2,0	30,8
17	3	1,72±0,67	—	—
23	2	0,5 and 1,4	31,0	15,0
30	1	1,0	3,0	3,0
40	2	0,75 and 0,68	6 and 26,0	11,09
50	1	0,56	6,0	3,0

preparation. Extensive areas of hematopoietic cells were arranged around the zone of necrosis: Cells of the granulocytic series formed small (10-15 cells at different stages of maturity) groups, uniformly distributed throughout the explant; megakaryocytes were rare. Mitotic figures, on the other hand, were numerous. Most cells belonged to the erythroid series (Fig. 1a).

During cultivation (14th-30th day) the number of hematopoietic cells (of all three series) increased in the explants: Myeloid cells were represented mainly by mature forms located in the upper layer of what was now the stratified explant. Large groups of erythroid cells formed extensive continuous areas (Fig. 1b).

In the late stages of cultivation (toward the 40th-50th day) the relative proportions of the different types of cells changed: The number of fibroblast-like cells increased both at the edge of the zone of growth and in the center of the explant. The hematopoietic cells became smaller and some of them died: There were many macrophages filled with hemosiderin. Small groups of erythroid cells could be seen on the surface of the explant (Fig. 1c). There were virtually no dividing cells. The cellular composition of the explants at the various stages of cultivation are given in Table 1.

The total number of living cells and the number of CFU_c in the cultures were studied from the 1st to the 50th day (Table 2).

During the first day of cultivation a decrease was observed both in the total number of cells and in the number of CFU_c. On the fifth day of cultivation the number of CFU_c in the explant increased, but did not reach its initial level, and later it decreased until the 50th day. The total number of living cells also fell at this time, and by the 50th day hematopoiesis was virtually exhausted. The number of CFU_c decreased more than the number of living cells (Table 2).

The results thus indicate that hematopoiesis of erythroid type can continue for a long time (over 1.5 months) in organ cultures of human embryonic liver. In cultures of mouse embryonic liver the erythroid cells were observed [3] to disappear by the 10th-14th day of cultivation, whereas the cells of the myeloid series persisted for a long time.

Differences in the type of hematopoiesis during cultivation of mouse and human embryonic liver may be associated with species differences. The possibility cannot be ruled out that the conditions of cultivation of mouse embryonic liver were inadequate to maintain erythroid hematopoiesis. Human embryonic liver cells may also be more sensitive to the presence of exogenous erythropoietin in the nutrient medium. Another possibility is that human embryonic liver cells can produce their own erythropoietin.

Research into the study of erythroid cells in organ culture of human embryonic liver is continuing at the present time.

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CHARACTERISTICS OF HEMATOPOIESIS IN NUDE MICE

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The state of the bone marrow, peripheral blood, and spleen was studied from the age aspect in nude mice, characterized by the presence of the recessive nu mutation. In athymic homozygous (nu/nu) individuals, various features were found in the blood system that distinguished them from heterozygous (nu/+) animals: a low lymphocyte count in the peripheral blood, bone marrow, and spleen, inhibition of erythropoiesis in the bone marrow, and hyperplasia of erythroid elements in the spleen.

KEY WORDS: *nude mice; peripheral blood; bone marrow; thymus; spleen.*

The question of the role of the thymus in hematopoiesis has not yet been fully explained. A very interesting aspect of this problem is the study of nude mice, which were described previously [1]. These mice are characterized by the almost complete absence of hair, as the result of a recessive nu mutation. Homozygous (nu/nu) individuals are also distinguished by absence of the thymus and by certain other features [3]. The leukocyte

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