PROLIFERATIVE ACTIVITY OF HEMATOPOIETIC STEM CELLS IN LONG-TERM CULTURES OF MOUSE EMBRYONIC LIVER

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The proliferative activity of hematopoietic stem cells was studied by the "thymidine suicide" method in organ cultures of mouse embryonic liver. Hematopoiesis was found to continue in these cultures for 2 months. The rate of proliferation starts to fall off only on the 50th day of culture; at the same time there is a sharp decrease in the number of residual hematopoietic stem cells in the cultures.
KEY WORDS: embryonic liver; hematopoiesis in culture; colony-forming units; rate of proliferation.

It is only recently that hematopoiesis has been successfully maintained in culture. The first reports of the preservation of hematopoiesis in organ cultures of embryonic mouse liver appeared only in 1970.

Hematopoietic stem cells, or colony-forming units (CFUs), were shown to be self-supporting in such cultures. On incubation of the culture for 24 h with a cytotoxic concentration of vinblastin, many of the CFU die.

Since vinblastin acts only on cells in a state of mitosis it was concluded that the CFU in such cultures proliferate for at least 17 days [4]. However, this conclusion is not sufficiently accurate. During the 24 h of action of vinblastin substantial death of morphologically distinguishable hematopoietic cells of the proliferative pool of the hematopoietic tissue takes place in the culture. Theoretically it can be imagined that this death of the more mature cells must involve a feedback mechanism – proliferation of CFU, which did not take place before the addition of the vinblastin, must begin.

The proliferation of CFUs in organ cultures of mouse embryonic liver was therefore studied by the "thymidine suicide" method (incorporation of H^3-labeled thymidine into cells in the S-period of the cell cycle). The "thymidine suicide" method, because of the short duration of the procedure (20-23 min), can not induce effects such as those that appear under the influence of vinblastin. Recent progress in culture techniques has made it possible to increase the life of the culture to 60 days.

EXPERIMENTAL METHOD

Cultures of the liver of 17-day CBA mouse embryos were set up. Organ cultures were grown from fragments of the liver [1]. The proportion of proliferating CFUs was determined by the "thymidine suicide" method [3] in the modification adopted in the author's laboratory [2]. Cells of the cultures were suspended in Hanks' solution and incubated with thymidine-H^3 of high specific activity (21.55 Ci/mmole) in a concentration of 100 μCi/ml with an exposure of 20-23 min. After incubation the cells were washed in 100 volumes of cold medium TS-199. The number of CFUs in the cell suspension was determined by the method of Till and McCulloch [5].

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TABLE 1. Proliferative Activity of Hematopoietic Cells of Embryonic Liver Cultures (M ± m)

Duration of cultivation (days)	No. of expts.	No. of living cells per liver, $\cdot 10^6$	No. of CFUs per liver	Proliferative activity (% of "thymidine suicide")
0	2	21,0±3,0	2513±104,0	36,8
3	3	3,96±0,58	600±30,5	45,8
4	4	5,7±0,30	832±186,0	48,1
7	3	2,8±0,95	891±56,0	53,2
10	3	2,2±1,0	350±120,0	58,3
14	3	0,85±0,29	158±22,2	45,6
17	3	1,73±0,08	247±45,0	51,4
24	3	0,98±0,20	325±52,0	37,5
31	2	0,77±0,73	150±12,0	41,6
40	2	1,01±0,49	312±78,0	33,1
50	1	0,19	50	24,5
60	1	0,38	26	10,9

EXPERIMENTAL RESULTS

The results are given in Table 1. Clearly at all times of cultivation colony-forming units were present in the explants. From the 10th to the 40th day of cultivation their number fluctuated very slightly. The results obtained with thymidine-H^3 show that the CFUs did not simply survive in culture, but proliferated steadily in it, for at any time until 1.5 months more than one-third of all the CFUs were in the period of DNA synthesis.

After 2 months in culture the proliferative activity of the CFUs declined, and there was a parallel sharp decrease in the number of CFUs remaining in the culture. This suggests that the life span of the CFU in culture quickly ceases.

An organ culture of embryonic liver is thus a system in which continuous proliferation and differentiation of CFUs take place. These processes are balanced in the culture so that the total number both of CFUs and of their differentiated progenies remain in a state of dynamic equilibrium for a long time. Consequently, factors stabilizing hematopoiesis in the explants operate in culture. The organ culture of embryonic liver is thus a convenient system with which to study the mechanism of regulation of hematopoiesis and, in particular, the factors determining the probability of proliferation or differentiation of hematopoietic stem cells.

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