DIFFERENTIATION OF HEMATOPOIETIC CELLS IN ORGAN CULTURES

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Organ cultures of mouse embryonic liver were grown on Millipore filters. On the 4th-18th day of cultivation the proliferative pool of hematopoietic tissue in the cultures was determined by addition of thymidine-H^3 and some temporal parameters of differentiation of its cells were studied.

During cultivation of the liver of 16-20-day mouse embryos in organ cultures on Millipore filters, hematopoiesis continues for a long time, up to 24-25 days [4, 6, 3]. The intensity of proliferation of the hematopoietic tissue in embryonic liver cultures correlates with the synthetic activity of its parenchyma, where fetal serum protein (αf-globulin) is synthesized [4, 6]. Besides differentiated hematopoietic cells, a line of hematopoietic stem cells capable of giving rise to large hematopoietic colonies in the spleen of irradiated mice [1] is maintained in the cultures. During the first 8-10 days of cultivation, cells of the erythroid and myeloid series are present in the explant, but later only myeloid hematopoiesis is maintained, including the formation of megakaryocytes.

The composition of the hematopoietic tissue in cultures on different days after explantation has been studied previously [1].

The object of the present investigation was to study proliferative activity of the cultures, the time taken by the cells to pass through the prolifierative and nonproliferative sections of the population, and the maturation time of the hematopoietic cells. These results were obtained by adding thymidine-H^3 to the culture medium.

EXPERIMENTAL METHOD

Organ cultures were set up as described previously [2]. Pieces (measuring 2 mm) of liver from 17-20-day CBA mouse embryos were placed on the surface of AUFS Millipore filters (pore size 0.6-0.9 μ) and grown above a liquid nutrient medium consisting of 70% medium No. 199, 15% young calf serum, 5% calf embryonic serum, and 10% chick embryonic extract. To each 100 ml medium, 7-10 ml vitamin C, 400 mg glucose, 20 mg Na-β-glycerophosphate, 20 mg L-glutamine, and 5000 units each of penicillin and streptomycin were added. The gas phase consisted of air. The medium was changed every 2-3 days. On the 4th-17th days of cultivation, thymidine-H^3 was added to the medium in a concentration of 1 μCi/ml, and 24 h later impressions were taken from the cultures and fixed with methyl alcohol, coated with photographic emulsion, exposed, and developed by the usual method [5]. The autoradiographs were then stained by the Romanowsky method. In a special series of experiments the thymidine-H^3 was present in the medium of the 7-day cultures for 1, 3, 7.5, 19, and 24 h before fixation.

To determine the formula of the hematopoietic tissue, 800-1200 cells were counted in the films. The percentage of labeled and unlabeled cells was counted separately among the myeloblasts, promyelocytes,

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TABLE 1. Formula of Myeloid Hematopoiesis in 7-day Cultures of Embryonic Liver and Change in the Labeling Index after Addition of Thymidine-H^3 to Nutrient Medium for 1, 3, 7.5, 19, and 24 h

	Formula (in %)	Labeling index				
		1 h	3 h	7½ h	19 h	24 h
Myeloblasts	2	60	65	90	100	100
Promyelocytes	7	66	75	97	100	100
Myelocytes	26	51	63	90	100	100
Metamyelocytes	13	30	53	73	90	100
Stab cells	40	0	2	7	64	79
Polymorphs	12	0	0	0	24	54

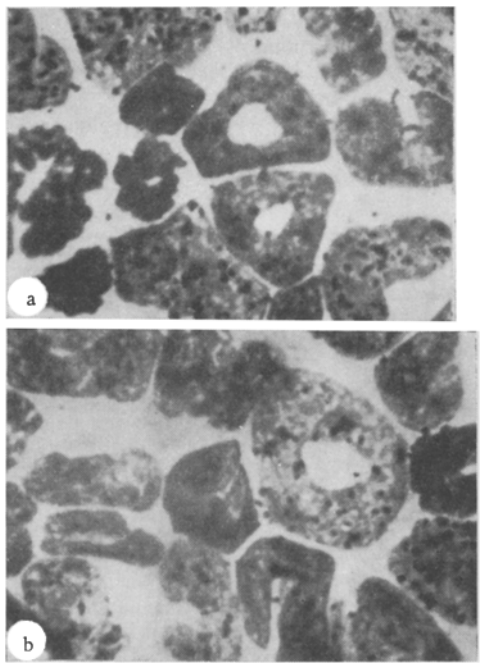

Fig. 1. Autoradiographs of 14-day (a) and 18-day (b) cultures of embryonic liver and 24 h after addition of thymidine-H^3. Label in myeloid cells differing in their degree of maturity. Azure-eosin, 60×.

myelocytes, metamyelocytes, stab cells, and polymorphonuclear leukocytes.

EXPERIMENTAL RESULTS

From the 4th to the 18th days of cultivation, i.e., at all times of the investigation, the hematopoietic tissue in the explant showed high proliferative activity. After cultivation for 24 h in medium containing thymidine-H^3, the isotope was incorporated into 100% of the myeloblasts, promyelocytes, myelocytes, and metamyelocytes and into 71-78% of the stab cells. The percentage of labeled polymorphs varied from 50 to 73.

The formula of the hematopoietic tissue and change in the index of labeling among each category of myeloid cells in the 7-day cultures are given in Table 1 in relation to the period of exposure with thymidine-H^3.

The results show that in 4-18-day organ cultures of embryonic liver, the myeloid cells exhibited high proliferative activity; all categories of myeloid cells capable of proliferation were in the pool (Fig. 1), which constituted virtually 100% of the cells of the proliferative section of the population. The fact that 100% of the myeloblasts, promyelocytes, and myelocytes in the 7-day cultures were labeled after 19 h indicates that the duration of the life cycle of all these cells in the organ culture was less than 19 h, and in the case of metamyelocytes, less than 24 h.

The first labeled stab cells appeared after 3 h, and after 24 h their labeling index was 79%. Consequently, stab cells did not proliferate in the organ cultures and their lifespan exceeded 24 h. The time of differentiation from metamyelocyte to stab cell was evidently about 3 h, because it was at this time that the first labeled stab cells appeared. By the same argument, the shortest time of differentiation from metamyelocyte to polymorph exceeded 7.5 h, and half the lifespan of the polymorphs during organ cultivation was about 10 h. The fact that during the first hour of cultivation with thymidine-H^3 only unlabeled mitoses were observed, while in 3-h cultures a certain number of labeled mitoses appeared, suggests that the postsynthetic phase in myeloid cells in culture lasts about 3 h.

After cultivation for 1 h in the presence of thymidine-H^3, a considerable proportion (30%) of the metamyelocytes in the 7-day cultures were labeled. However, not all mitoses contained label at this time. Hence it follows that labeled metamyelocytes evidently appear in organ cultures either by differentiation of labeled myelocytes, unaccompanied by cell division, or by the proliferative activity of the metamyelocytes.

These results, on the whole, are evidence of intensive proliferation and differentiation of myeloid cells during organ cultivation.

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