CHARACTERISTICS OF BONE MARROW PRECURSORS OF FIBROBLAST-LIKE CELLS WITH REFERENCE TO THEIR INCORPORATION OF THYMIDINE-H^3

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After pulse and continuous (26-72 h) labeling of monolayer cultures of guinea-pig bone marrow with thymidine-H^3 the overwhelming majority of fibroblasts are unlabeled (index of labeled fibroblasts at saturation 2-5%). Under these conditions the proportion of labeled cells is always several times higher among the histiocytes than among the fibroblasts. In bone marrow cultures from a 6-day animal about 15% of fibroblasts were found to be labeled 72 h after saturation with the isotope. Judging from the intensity of incorporation of thymidine-H^3, the precursors of the fibroblast-like cells evidently belong to the category of bone marrow cells which, in a state of equilibrium, are at rest or have a very long life cycle.

The multiplication and development of histiocytes and fibroblast-like cells in cultures of hematopoietic tissue were described in the earliest studies of cell culture in vitro [3, 4, 8]. It has recently been shown that the histocytes of hematopoietic tissue are the progeny of the hematopoietic stem cell [10, 12-14]. On the other hand it has been shown that fibroblast-like cells in monolayer cultures of bone marrow grow as discrete foci which, as analysis of chromosome markers and the distribution of cells labeled with thymidine-H^3 has shown, are cell clones [1, 5]. Colonies of fibroblasts are reformed in subcultures taken from the primary cultures, i.e., the population of stromal fibroblast-like elements is maintained [5]. The relationship between this line of bone marrow cells and the remaining cells of the hematopoietic tissue, especially the hematopoietic stem cells, is of evident interest. According to some observations, subcutaneous fibroblasts in bone marrow radiochimeras at long intervals after the formation of the chimerism, are of recipient origin [6, 11], i.e., they are capable of supporting themselves for a long time under these conditions. The number of precursor cells for colonies of fibroblasts in the initial suspension of bone marrow cells is very limited (of the order of $5 \cdot 10^{-4}$), and for this reason it is evidently not yet possible to identify these cells morphologically. The study of those properties of colony-forming cells which might shed light on their origin is therefore of great interest.

The object of the present investigation was to determine whether the bone marrow cells which are precursors of the fibroblasts growing in cultures belong to the same category as the bone marrow cells which synthesize DNA in vivo.

EXPERIMENTAL METHOD

Bone marrow for explantation was taken from the femora of guinea-pigs aged 6 days, 2 weeks, and 3 months. Thymidine-H^3 (4.3 mCi/mmole) was injected intraperitoneally by the following schemes: in a total dose of 1 mCi/g three times in the course of 1 h (conventional pulse labeling), in a total dose of 0.25 mCi/g for 3 days at intervals of 2 h, and in a total dose of 1 mCi/g for 26 h at intervals of 2 h. The animals were sacrificed 20 min after the last injection of the isotope. The method of preparing the bone marrow

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TABLE 1. Percentage of Labeled Cells in Bone Marrow Cultures

Age of donor	Conditions of administration of thymidine-H^3 to donor	Labeled fibroblasts (in %)	Labeled histiocytes (in %)
6 days	For 72 h at intervals of 2 h	15±0,6	89,8±0,2
2 weeks	Same	2,2±1,3	56,8±1,7
3 months	Pulse labeling	0	22,2±2,3
3	For 26 h at intervals of 2 h	4,6±3,2	54,5±2,5

suspensions and the conditions under which they were cultivated ($2 \cdot 10^6$-$2 \cdot 10^7$ cells per flask) were described previously [2]. Unlabeled thymidine was added in a concentration of 25 μg/ml at the time of explantation and daily thereafter.

On the 3rd-6th day after explantation the cultures were washed in physiological saline and fixed in 96° alcohol. After treatment in perchloric acid the specimens were washed for 1 h in tap water, dried, coated with type M emulsion, and exposed in darkness for 10 or 30 days. The developed specimens were stained with Carazzi's hematoxylin or azure-eosin. In each experiment 400-600 fibroblasts and 400 histiocytes were counted. Foci of fibroblasts containing more than eight cells were not counted because of possible dilution of the label. Cells with more than five grains of silver above the nucleus were regarded as labeled.

EXPERIMENTAL RESULTS

Almost 90% of the histiocytes but only 15% of the fibroblasts were labeled in monolayer cultures of bone marrow of a 6-day old guinea-pig after saturation for 3 days with thymidine-H^3 (Table 1).

In material taken from a 2-week old guinea pig and after administration of the isotope under the same conditions there were far fewer labeled histiocytes and, in particular, fibroblasts (56.8 and 2.2% respectively), but even so more than half of the histiocytes contained the label.

In adult (3 months) guinea-pigs virtually no labeled fibroblasts could be found after pulse labeling, whereas 22% of the histiocytes contained the label. After saturation for 24 h the proportion of labeled histiocytes rose to 54.5%, while among the fibroblasts only 4.6% were labeled.

The relatively small number of precursors of fibroblasts makes it difficult to identify them in the bone marrow films from the animals, even after saturation labeling. However, comparison of the proportion of labeled histiocytes and fibroblasts indicates that their immediate precursors belong to different categories of bone marrow cells. The results for incorporation of thymidine-H^3 by fibroblast-like cells given above suggests that their precursors in vivo belong to the pool of DNA-synthesizing cells, for the possibility of reutilizing the label by fibroblasts in bone marrow cultures is virtually excluded if unlabeled thymidine is added to the culture medium [1].

Since more than 90% of the fibroblasts are in the proliferative pool after cultivation for 60 h [1], judging from their entry into the S-period, and assuming that the progeny of the labeled and unlabeled precursors proliferate at equal rates in the cultures, the proportion of labeled fibroblast-like cells can be considered to reflect reliably enough the proportion of their DNA-synthesizing precursors.

In the course of administration of thymidine-H^3 for 3 days all cells in which $T - T_S$ is equal to this time must be labeled (T is the duration of the life cycle and T_S the duration of the S-period). For most cells types studied $T_S \sim {}^1/_3$ T, so that $T - T/3 = 3$ days, i.e., all cells for which T = 4.5 days must be labeled, as well as some cells with a longer life cycle. Under these conditions of administration of the isotope, it was found that in the 6-day guinea pig only a small proportion of the population of fibroblast precursors about 15%) was labeled, and with age this proportion fell rapidly to 2-5%, while pulse labeling revealed no labeled precursors of the fibroblasts whatever.

It thus follows that the overwhelming majority of precursors of the fibroblast-like cells are in a state of equilibrium (in the normal adult organism) at rest or have a very long life cycle. Most of these cells in the equilibrium state do not divide. These properties are characteristic of stromal cells, 3-5% of the small lymphocytes of the bone marrow, and hematopoietic stem cells [7, 9]. By contrast with precursors of the

fibroblasts, which also are evidently included in this fraction, the precursors of histiocytes belong to the category of bone marrow cells which proliferate actively in the adult organism.

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