THE KINETICS OF PROMONOCYTES AND MONOCYTES IN THE BONE MARROW

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The monocytes of the peripheral blood derive from a precursor cell in the bone marrow (1). The morphological and functional characteristics of these precursor cells, which are termed promonocytes, have been described in the preceding paper (2).

Initially, the term promonocytes was given to all mononuclear phagocytes of the bone marrow because of their labeling characteristics as determined in 48-hr cultures (1). In a subsequent study the mononuclear phagocytes in the bone marrow were classified, on the basis of a 6 hr culture period, into two cell types, promonocytes and monocytes, the differentiation being based on morphological and functional criteria.

To investigate the validity of this classification, i.e. whether the promonocytes demonstrated in 6-hr cultures are indeed the progenitors of the monocytes, the mononuclear phagocytes of the bone marrow were labeled in vitro and in vivo with tritiated thymidine. The present paper concerns itself with these labeling studies and describes the kinetics of the promonocytes and monocytes in the bone marrow compartment.

Materials and Methods

Animals.—This study was done in specific pathogen-free Swiss mice, originally deriving from the strain held at The Rockefeller University, New York, and raised at the Central Institute for the Breeding of Laboratory Animals, TNO, Bilthoven, The Netherlands. Only male mice with a body weight between 25 and 30 g were used.

Bone Marrow Cultures.—The methods used to obtain bone marrow cell suspensions and to culture these cells have been described in detail elsewhere (1). A cell suspension was made from the bone marrow of both femurs of a mouse. The culture medium consisted of medium 199 (Microbiological Associates, Inc., Bethesda, Md.), 20% newborn calf serum (Colorado Serum Co., Denver, Colo.), 200 units/ml penicillin G and 50 μg/ml streptomycin. All cultures were prepared in Leighton tubes. The cell suspensions were first incubated for 2 hr to allow the cells to attach to the cover slip. The supernatant was then removed, and the cell layer on the cover slip was washed three times by vigorous shaking in 1.5 ml medium 199. The cells on the cover slip were then incubated again with 2 ml of culture medium for an additional 4, 22, or 46 hr; for the 48-hr cultures, the medium was renewed after 24 hr. After total incubation times

of 6, 24, or 48 hr, the cells on the cover slips were washed again three times, quickly air dried, and fixed for 30 min in absolute methyl alcohol.

Peripheral Blood Monocytes.—Monocytes of the peripheral blood were studied in smears prepared from tail-vein blood. The slides were fixed in absolute methyl alcohol for 30 min.

Labeling with Thymidine-^3H.—The in vitro labeling of the mononuclear phagocytes of the bone marrow was performed by incubating the cells for a given period in the culture medium, which contained 0.1 μCi/ml methyl thymidine-^{3}H (specific activity 6.7 mCi/mmole, New England Nuclear Corp., Boston, Mass.). The culture medium used to flush the femur shafts already contained thymidine-^{3}H. After the initial incubation period of 2 hr, the cover slip preparations were washed and again cultured. The cells on the cover slips were incubated in the presence of thymidine-^{3}H for a total period of 2, 6, 24, or 48 hr, after which the cover slips were washed five times with medium 199 to ensure the removal of all thymidine-^{3}H. According to the requirements of the experiment, the slides were then reincubated in the nonradioactive culture medium or quickly air dried, fixed with absolute methyl alcohol, and prepared for radioautography.

In vivo labeling was done by the administration of one intravenous injection of thymidine-^{3}H, in a dose of 1 μCi/g body weight. The bone marrow was harvested from 2–96 hr after the injection. For each animal, one of the duplicate bone marrow cultures was incubated for 6 hr and the other for 48 hr, as described elsewhere (1). After incubation, the cover slips were washed, rapidly air dried, fixed in absolute methyl alcohol, and prepared for radioautography.

Serial injections of thymidine-^{3}H were also applied to a group of mice to determine the DNA-synthesis time of the promonocytes. These mice received 1, 2, or 3 intravenous injections of thymidine-^{3}H in a dose of 1 μCi/g body weight, with a 2 hr interval, at the time points 0, 2, and 4 hr. Bone marrow was taken 1 and 2 hr after each injection and cultured for 6 hr. The labeling index for each time point represents the mean of values for three mice.

Radioautography.—Radioautography was performed with the Ilford Nuclear Research Emulsion K5, in gel form (Ilford Ltd., Ilford, Essex, England) (1). The developed slides were stained with Giemsa stain (1). Bone marrow cultures were stained for about 20 min and blood smears for about 90 min.

All cells with three or more silver grains over the nucleus were considered as labeled, since background levels on preparations from nonlabeled animals showed less than three grains per nucleus.

At each time point, at least 200 mononuclear phagocytes were counted in duplicate bone marrow cultures deriving from one mouse. The labeling index for each time point in the graphs or tables represents the mean value of at least two mice but in many cases of four to eight mice.

The total percentage of labeled cells (Table I and Fig. 4) was calculated by using the labeling indices and the percentage distribution of promonocytes and monocytes on the same slide.

The mean grain count at each time point was calculated from the grain counts determined in 100 labeled cells.

Determination of the DNA Content of the Promonocytes and Monocytes.—Bone marrow cell suspensions were cultured for 6 hr, washed, air dried, and fixed with absolute methyl alcohol. After the cells on the cover slip had been stained by the Feulgen reaction (3), the DNA content of the nuclei was determined ultramicrospectrophotometrically (Zeiss Ultramicrospectrophometer I, Carl Zeiss, Loberkochen, W. Germany [4]). As a reference for the diploid value, the DNA content of the polymorphonuclear cells on the same slide was used.

Identification of the Cells.—The criteria used for the morphological differentiation of the mononuclear phagocytes of the bone marrow into promonocytes and monocytes has been discussed in the preceding paper (2). Promonocytes have a diameter of 14–20 μ, a basophilic cytoplasm, and a relatively large indented or folded nucleus; monocytes have a diameter of 11–14 μ, a pale-blue cytoplasm, and an indented horseshoe-shaped or reniform nucleus. The ratio between promonocytes and monocytes is about 1:5 (2).

RESULTS

In Vitro Labeling of Promonocytes and Monocytes of the Bone Marrow.— After incubation of bone marrow cells for 2 hr in a culture medium containing thymidine-^{3}H, and reincubation in a nonradioactive medium for 4 hr, 47.6% (range 38.5–66.6%) of the promonocytes were labeled, but no labeled monocytes were present (Table I). Fig. 1 shows a labeled promonocyte which was allowed to phagocytize *Staphylococcus albus* (2). When the bone marrow cells were incubated during the entire 6 hr culture period in the presence of thymidine-^{3}H, the monocytes were again unlabeled, and 54.0% (range 39.0–63.0%) labeled promonocytes were found; in the 24-hr cultures 50.3% (range 40.5–58.0%) of the promonocytes were labeled (Table I). Labeling of a small percentage of monocytes (range 0–6.9%) occurred only in bone marrow cultures incubated for 24 hr (Table I).

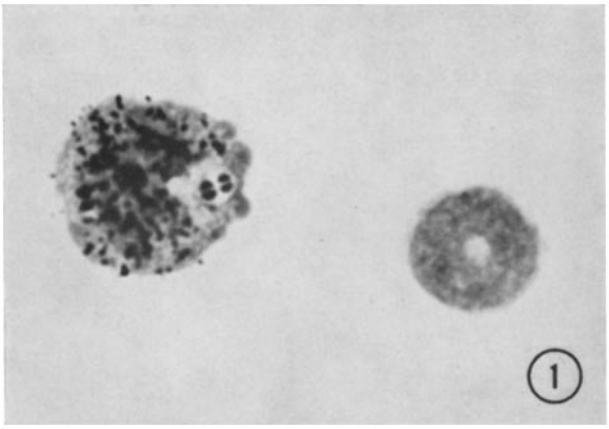

FIG. 1. A promonocyte labeled in vitro and a doughnut cell. The promonocyte has phagocytized *Staphylococcus albus*. × 1200.

After a culture period of 48 hr, the classification into promonocytes and monocytes could not be made because all the cells had the morphology of macrophages (2). With incubation during the total culture period of 48 hr in a medium containing thymidine-^{3}H, 28.2% (range 19.5–41.0%) of the mononuclear phagocytes were labeled (Table I). When the bone marrow cells initially incubated in the radioactive medium for only 2 or 6 hr were cultured for 48 hr, 22.5% (range 18.5–26.5%) and 20.3% (range 19.5–21.5%) of the mononuclear phagocytes were labeled respectively.

To compare the percentages of labeled mononuclear phagocytes found after 48 hr of incubation with those of the 6- and 24-hr cultures, the total percentage of labeled cells from these cultures was calculated, taking into account the percentage distribution of the promonocytes and monocytes on

the same slide. These mean values ranged from 10.8 to 15.4%, which is lower than the labeling indices of the 48-hr cultures (Table I).

The mean grain count of the promonocytes labeled in vitro for 2 hr was 44.3 grains per cell after a total incubation time of 6 hr. This value decreased to 31.4 grains per cell after an incubation period of 48 hr (Table II). Since no thymidine-^{3}H was present after the first 2 hr of incubation, the labeled cells found at 48 hr may be considered to derive from the initially labeled promonocytes. Similar results were obtained when the bone marrow cells were incubated for 6 hr in the presence of thymidine-^{3}H. The mean grain counts of the pro-

TABLE I

In Vitro Labeling of Mononuclear Phagocytes of the Bone Marrow

Thymidine-^{3}H in medium*	Total culture time	Labeled cells		
		Promonocytes	Monocytes	Total
hr	*hr*	%	%	%
2	6	47.6	0	10.8‡
2	24	45.7	1.5	13.7‡
2	48			22.5§
6	6	54.0	0	12.6‡
6	24	50.5	2.0	15.4‡
6	48			20.3§
24	24	50.3	2.4	15.8‡
48	48			28.2§

* 0.1 μCi/ml thymidine-^{3}H.

‡ Values calculated from the labeling indices and percentage distribution of promonocytes and monocytes.

§ At this time point all cells are morphologically macrophages.

monocytes were found to be higher, but this also dropped with longer incubation periods in a nonradioactive medium (Table II).

The results of these in vitro labeling studies show that the promonocytes incorporate thymidine-^{3}H, which indicates that these are dividing cells. However, the monocytes of the bone marrow did not synthesize DNA. This is in accordance with earlier results, which demonstrated that the monocytes of the peripheral blood were not labeled after incubation for 24 hr in the presence of thymidine-^{3}H (1). If the absence of in vitro labeling of the monocytes reliably reflects the state of DNA synthesis of these cells in vivo, then monocytes must be nonproliferating cells. The small percentage of labeled monocytes found in the 24-hr cultures probably originated from promonocytes dividing in vitro, as discussed below.

The labeling index of the promonocytes appears to be independent of the

TABLE II

Mean Grain Counts of Promonocytes Labeled In Vitro

Incubation with thymidine-^{3}H*	Total incubation time	Mean grain count
hr	*hr*	
2	6	44.3
2	24	35.1
2	48	31.4
6	6	90.6
6	24	61.9
6	48	53.1

* In medium with 0.1 μCi/ml thymidine-^{3}H.

time of incubation with thymidine-^{3}H. However, after an incubation period of 48 hr the total percentage of labeled cells increased 1.5–1.8 times as compared to the calculated values for the 6- and 24-hr cultures (Table I). This significant increase ($P < 0.005$) could be due to the death of unlabeled cells or to division of the labeled promonocytes. The decrease of the mean grain counts of the labeled cells with time of incubation indicates that at least some of the promonocytes divide during incubation in vitro.

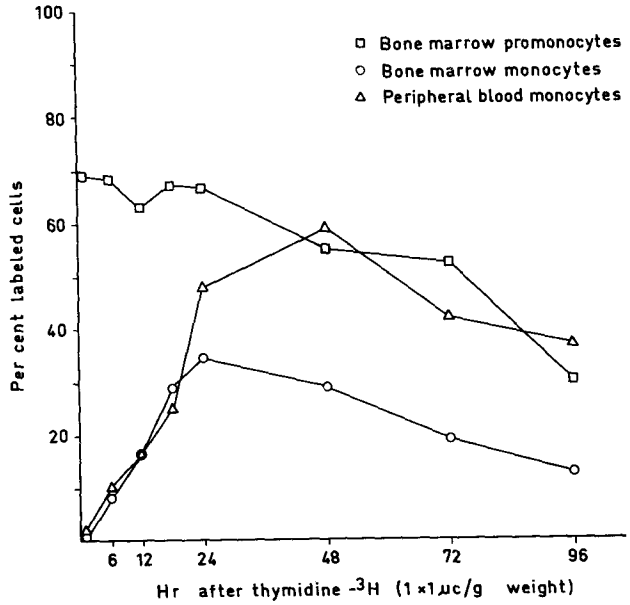

FIG. 2. Labeling of promonocytes and monocytes of the bone marrow and of monocytes of the peripheral blood after one intravenous injection of thymidine-^{3}H.

Determination of the DNA Content in the Nuclei of the Promonocytes and Monocytes.—The microspectrophotometric determination of the DNA content of the nuclei of the monocytes demonstrated that all monocytes have a diploid amount of DNA. This result agrees with the absence of in vitro labeling of these

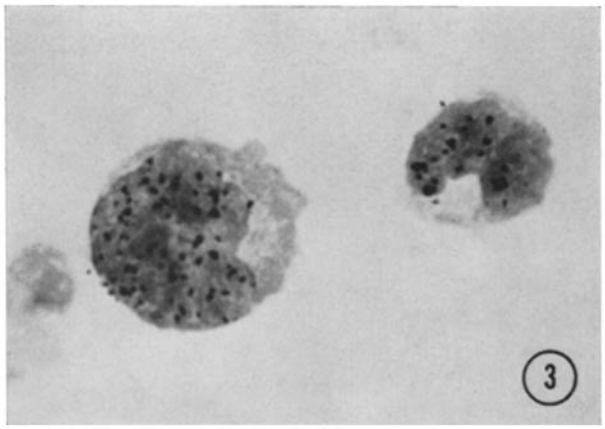

FIG. 3. A promonocyte and a monocyte of the bone marrow after in vivo labeling. × 1200.

TABLE III

Mean Grain Counts of Promonocytes and Monocytes of the Bone Marrow Labeled In Vivo

Time after thymidine-^{3}H*	Mean grain count	
	Promonocytes	Monocytes
hr		
2	21.6	
6	19.7	13.9
12	10.7	9.8
24	9.9	9.5
48	6.1	8.2
72	5.9	6.1

* 1 × 1 μCi/g body weight intravenously.

cells with thymidine-^{3}H. In contrast, 80% of the promonocytes had a DNA content that was more than diploid and less than or equal to the tetraploid value. These DNA values agree with the in vitro and in vivo labeling data, which showed that 50–70% of the promonocytes incorporate thymidine-^{3}H.

In Vivo Labeling of Promonocytes and Monocytes of the Bone Marrow and Peripheral Blood Monocytes after one Injection of Thymidine-^{3}H.—The in vivo labeling studies demonstrated that 2 hr after a single injection of thymidine-^{3}H, 68.7% of the promonocytes were labeled (Fig. 2). This labeling index remained

almost constant during the first 24 hr; thereafter, the percentage of labeled promonocytes decreased slightly to a value of 52.6% at 72 hr, and at 96 hr the labeling index had dropped to 29.3% (Fig. 2). Since promonocytes are dividing cells, this decrease of the labeling index at 96 hr can probably be ascribed to the fact that at later time points some proportion of the promonocytes, all of which derive from initially labeled cells, had grain counts below the 3 grain threshold and thus were not considered positive. Fig. 3 shows a promonocyte and a monocyte labeled in vivo.

The labeling index of the monocytes in the bone marrow 2 hr after the

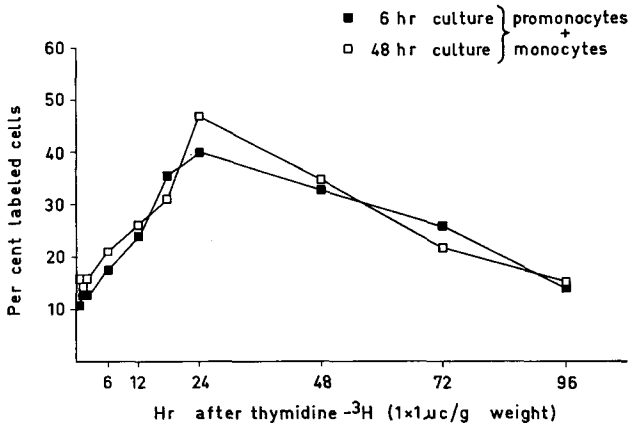

FIG. 4. Labeling of mononuclear phagocytes of the bone marrow (promonocytes and monocytes) observed in 6- and 48-hr cultures. The values of the 6-hr cultures were calculated from labeling indices and promonocyte to monocyte ratio at each time point.

thymidine-^{3}H injection was only 1%, and reached its highest value, i.e. 33.3% labeled monocytes, at 24 hr. Thereafter, this percentage decreased gradually to 12.3% at 96 hr (Fig. 2).

The labeling indices of the peripheral blood monocytes, determined in the same mice, are also presented in Fig. 2. This curve demonstrates that 2 hr after a single injection 2% of the monocytes were already labeled, and that the highest value (59%) was attained at 48 hr. These results agree with the findings in a previous study (1).

The mean grain counts of the labeled promonocytes and bone marrow monocytes (Table III) gave for the promonocytes a value of 21.6 grains per cell 2 hr after the thymidine-^{3}H injection. This value decreased with time, and at 72 hr amounted to 5.9 grains per cell. At 6 hr the mean grain count of the monocytes was 13.9 grains per cell, i.e. half the initial mean grain count of the

promonocytes, which may indicate that monocytes are derived from promonocytes after division.

At each time-point, a second bone marrow culture of the same mouse was also incubated for 48 hr. After this period of incubation, the mononuclear phagocytes of the bone marrow can no longer be classified as promonocytes and monocytes because morphologically all the cells are macrophages (1, 2). In these cultures, 15% of the mononuclear phagocytes were labeled 2 hr after thymidine-^{3}H injection. The labeling index increased with time until the highest value, i.e. 47% labeled cells, was reached at 24 hr. Thereafter, the labeling index decreased to 15% at 96 hr (Fig. 4). Similar values were found in earlier experiments (1).

TABLE IV

Percentage of Labeled Promonocytes and Monocytes after Serial Injections of Thymidine-^{3}H

Thymidine-^{3}H injection* at time point	Bone marrow cultured‡ at time point	Labeled cells	
		Promonocytes	Monocytes
hr	*hr*	%	%
0	1	71.3	0.3
0	2	68.2	1.1
0 + 2	3	80.1	2.4
0 + 2	4	83.4	2.8
0 + 2 + 4	5	90.1	10.5
0 + 2 + 4	6	92.8	11.0

* 1 μCi/g body weight intravenously.

‡ Cell suspensions incubated for 6 hr.

From both the data on the labeled promonocytes and monocytes in the 6-hr cultures and the ratio of promonocytes to monocytes for each slide, the total percentage of labeled cells was calculated for each time point. These percentages are shown in Fig. 4. Since these two curves coincide almost completely, there is excellent agreement between the percentages of labeled cells found in the 6- and 48-hr cultures. These results therefore provide additional support for the validity of the classification of the mononuclear phagocytes of the bone marrow into promonocytes and monocytes.

Both the total number of labeled monocytes formed after a single injection of thymidine-^{3}H and their distribution over the bone marrow and blood compartments can be calculated, since the size of these two pools can be determined (see Discussion: the monocyte pool in the peripheral blood amounts to 1.1×10^6 cells and the bone marrow contains 2.4×10^6 monocytes). At each time point, the number of labeled bone marrow monocytes will be the product of the labeling index and the number of monocytes in the bone marrow compartment ($I_{bm} \times N_{bm}$). The number of labeled monocytes released into the pe-

ripheral blood is at each time point the product of the labeling index and the total number of peripheral blood monocytes plus the number of monocytes that have already left the peripheral blood compartment ($I_{bl} \times N_{bl} + 0.6932/T\frac{1}{2} \int_0^t I_{bl} \times N_{bl}\, dt$) (Fig. 5). The total number of monocytes produced is the sum of the labeled monocytes in the bone marrow and the monocytes released nto the peripheral blood. Accordingly, during the first 18 hr after labeling the

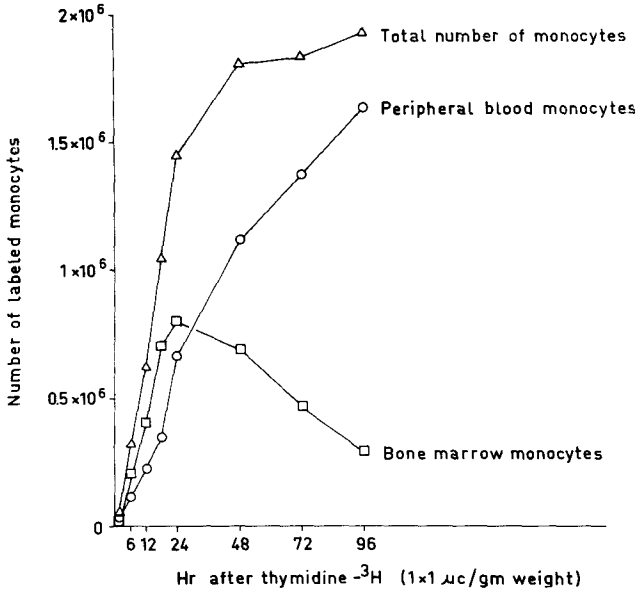

FIG. 5. Total production of labeled monocytes and their distribution over the bone marrow and blood compartments, after a single injection of thymidine-^{3}H. The number of labeled bone marrow monocytes is: $I_{bm} \times N_{bm}$. The number of labeled peripheral blood monocytes is: $I_{bl} \times N_{bl} + 0.6932/T\frac{1}{2} \int_0^t I_{bl} \times N_{bl}\, dt$. (I = labeling index; N_{bm} = total number of bone marrow monocytes; N_{bl} = total number of peripheral blood monocytes; $T\frac{1}{2}$ = 22 hr [1].) The total number of monocytes produced is the sum of the monocytes in the bone marrow compartment and the monocytes released into the peripheral blood.

number of labeled monocytes in the bone marrow compartment increases by 4.1×10^4 cells/hr and 2.0×10^4 monocytes/hour are released into the peripheral blood. However, in the period from 18–24 hr 5.2×10^4 labeled monocytes/hr are released into the peripheral blood and the increase of labeled bone marrow monocytes is only 1.6×10^4 cells/hr. After 24 hr the formation of labeled monocytes levels off (Fig. 5).

Serial Labeling with Thymidine-^{3}H for the Determination of the DNA-Synthesis Time and the Generation Time of Promonocytes.—Mice were labeled one, two, or three times by intravenous injection of thymidine-^{3}H at 2-hr intervals.

In the bone marrow samples harvested 1 and 2 hr after each injection, the labeling index of the promonocytes and monocytes was determined. For the promonocytes harvested 1 hr after the first injection of thymidine-^{3}H, this value was 71.3% (Table IV); 2 hr after the third injection, the index had increased to 92.8%. The initially very low (0.3%) percentage of labeled monocytes had increased to 11% 2 hr after the third injection.

The percentage of promonocytes entering DNA synthesis during the 2-hr periods between the injections can be determined from the increase of the labeling indices. For the calculation of the DNA synthesis time, the mean of the labeling indices of the promonocytes found 1 and 2 hr after the first injection of thymidine-^{3}H (69.8%) and the labeling index found 1 hr after the second injection (80.1%) were used. The increment of the labeling index during this 2 hr interval between the thymidine-^{3}H injections amounted to 10.3% (i.e. 5.15%/hr).

The DNA-synthesis time can be computed by dividing the initial labeling index by the increment of the labeling index per hour (5). The average DNA-synthesis time of the promonocytes is then $69.8/5.15 = 13.6$ hr. This approach is valid if the following criteria are satisfied: studies must be done under normal steady-state conditions, the interval between the thymidine-^{3}H injections must be shorter than the DNA-synthesis time, and the whole generation of dividing cells, i.e., the promonocytes, must be recognizable as one cytological class (4).

The generation time, i.e. the time between two consecutive divisions, can be computed by dividing the DNA-synthesis time by the initial labeling index (5). This gives an average generation time for the promonocytes of $(13.6 \times 100)/69.8 = 19.5$ hr.

DISCUSSION

Previous investigations indicated that the monocytes of the peripheral blood derive from a precursor cell in the bone marrow (1). In the preceding paper it was shown that in 6-hr cultures the mononuclear phagocytes of the bone marrow can be classified morphologically into two cell types, promonocytes and monocytes (2). The present study was performed to investigate the validity of the hypothesis that the promonocytes are the progenitors of the monocytes and to determine the kinetic characteristics of the promonocytes and the monocytes in the bone marrow compartment.

Proliferation Pattern of the Promonocytes.—The results of the in vitro labeling studies permit the conclusion that the promonocytes are proliferating cells, since about 50% of these cells are labeled after incubation with thymidine-^{3}H. However, under normal steady-state conditions the monocytes of the bone marrow appear to be nondividing cells, as was previously shown to be true

for blood monocytes (1). These conclusions are supported by the observation that the nuclei of the monocytes have a diploid amount of DNA, whereas the majority of the promonocytes have a DNA content that is more than diploid and less than or equal to tetraploid.

Further evidence supporting this conclusion has been provided by the in vivo labeling studies. During the first 24 hr after a single injection of thymidine-^{3}H, the promonocytes showed an almost constant and high labeling index. The labeling index of the monocytes of the bone marrow increased during the first 24 hr after the administration of thymidine-^{3}H, but the mean grain count remained almost constant during that period, being about half that of the promonocytes during the first 6 hr of the experiment. Since monocytes are nondividing cells, these findings indicate that the monocytes derive from the promonocytes after the latter have divided once.

Now let us consider the question of whether promonocytes are multiplicative cells, i.e. cells only capable of a rather limited number of mitoses, or are self-replicating cells, i.e., cells capable of continuous self-perpetuation (5). For the purposes of this discussion, we can take a model population of 1000 bone marrow mononuclear phagocytes; the total number of labeled promonocytes and bone marrow monocytes is calculated on the basis of the percentage distribution of the promonocytes and monocytes and their labeling indices at each time point. The total number of labeled peripheral blood monocytes corresponding to this model of the bone marrow population could also be calculated, since the relative size of the monocyte pool in the peripheral blood is known (see below). The results of these calculations (see Table V) show that the total number of labeled promonocytes remains constant during the first 24 hr after labeling, and that the total number of labeled bone marrow and peripheral blood monocytes increases with time.

2 hr after labeling, this model population will contain 110 labeled promonocytes. If the promonocytes were multiplicative cells, after the first division these 110 cells would give rise to 220 labeled monocytes having half the grain count shown by the promonocytes at 2 hr. Actually, these values were found 12 hr after labeling. At that time point the total number of labeled bone marrow plus peripheral blood monocytes (the latter being cells that have recently left the bone marrow) amounts to 215 cells. This would then indicate that the DNA-synthesis time, G_2-phase, and mitotic time together must be roughly 12 hr; this is compatible with the average DNA-synthesis time of 13.6 hr computed from the data obtained after repeated labeling with thymidine-^{3}H. (The minimum duration of the G_2-phase and mitosis must be 2 hr or less, since labeled monocytes were observed in the bone marrow and peripheral blood as early as 2 hr after labeling.) After one more division, which requires the full generation time of 19.6 hr, the total number of labeled monocytes can

be expected to have roughly doubled again, if monocytes having left the peripheral blood during that period are not taken into consideration. Actually, the total number of labeled bone marrow plus peripheral blood monocytes found at 24 and 48 hr lies just a little higher than the expected value. The labeled promonocytes observed at these time points must derive from cells of a preceding compartment, probably after division.

If promonocytes were self-replicating cells, after the first division there would be 110 promonocytes and 110 monocytes, both with half the mean grain count

TABLE V

The Total No. of Labeled Promonocytes, Bone Marrow Monocytes, and Corresponding Peripheral Blood Monocytes Computed for a Model Population of 1000 Mononuclear Phagocytes of the Bone Marrow

Hr after thymidine-^{3}H injection*	Promonocytes			Bone marrow monocytes			Peripheral blood monocytes		
	Total No. of cells	Percentage of labeled cells	Total No. of labeled cells	Total No. of cells	Percentage of labeled cells	Total No. of labeled cells	Total No. of cells	Percentage of labeled cells	Total No. of labeled cells
2	160	69	110	840	1	8	425	2	9
6	155	68	105	845	9	76	425	10	43
12	135	63	85	865	16	138	425	18	77
24	163	67	109	837	33	276	425	48	204
48	153	55	84	847	29	245	425	59	251

* 1×1 μCi/g body weight intravenously.

of that of the promonocytes at 2 hr. This is observed for the monocytes at 6 hr, but at that time point the mean grain count of the promonocytes is not halved and furthermore, the observed DNA-synthesis time of the promonocytes is more than 6 hr.

For both possibilities, an estimate of the DNA-synthesis time can be made from the flux of labeled promonocytes into the monocyte pool of the bone marrow and from there into the peripheral blood. If we consider the period between 2 and 12 hr for the model population (Table V), there would be an influx of 198 labeled monocytes during that interval, or 20 labeled monocytes per hour. If the promonocytes are multiplicative cells, after division both daughter cells would be monocytes. An influnx of 20 labeled monocytes per hour would then represent a flux into and out of DNA synthesis amounting to $20/2 = 10$ promonocytes per hr. Since the number of promonocytes in DNA synthesis amounts to 110 cells, the DNA synthesis time can be computed to be $110/10 = 11.0$ hr. If the promonocyte is a self-replicating cell, then numerically only one daughter cell of a divided promonocyte would become a monocyte and the other cell would remain a promonocyte. In that case, 20 promono-

cytes per hour would flow in and out of DNA synthesis. The DNA-synthesis time would then be 110/20 = 5.5 hr.

The results of a separate experiment in which repeated labeling of the mice with thymidine-^{3}H was applied, show that the average DNA synthesis time is 13.6 hr. This is compatible with the DNA-synthesis time of 11.0 hr estimated for the assumption that both daughter cells of the promonocyte are monocytes. If promonocytes were self-replicating cells the estimated DNA-synthesis time would have been half as long.

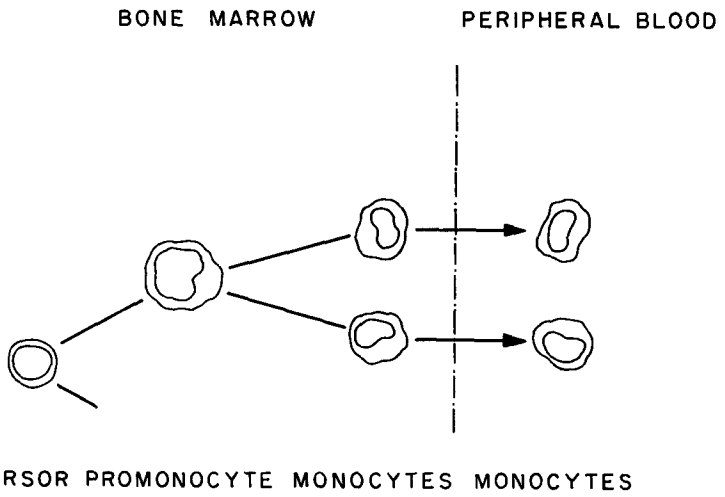

FIG. 6. Schematic representation of the proliferation of promonocytes and the formation of monocytes in the bone marrow.

It may therefore be concluded that the promonocytes are multiplicative cells; the division of one promonocyte gives rise to two monocytes (Fig. 6). In all probability the promonocytes divide only once. The pool of promonocytes is maintained by the influx of cells from a pool of precursor cells, whose identity is not yet known. These precursor cells probably do not stick to glass and would therefore escape detection with the methods currently used. The rather constant labeling index of the promonocytes during the first 24 hr after the administration of thymidine-^{3}H suggests, however, that the labeling index of the precursor cell must be rather similar to that of the promonocytes.

Size of the Promonocyte and Monocyte Pools in the Bone Marrow.—We may now consider the size of the promonocyte and monocyte pools in the bone marrow and of the monocyte pool in the peripheral blood. Since mouse blood contains 350 monocytes per mm^3 (6) and the blood volume of mice weighing 25

g is about 3 ml (7), the total number of monocytes in the peripheral blood must amount to 10.5×10^5 cells. From this value, the total number of monocytes in the bone marrow can be calculated if all monocytes formed in the bone marrow are assumed to enter the peripheral blood. Since the average transit time of the monocytes in the peripheral blood is 32 hr (1), under normal steady-state conditions the entry of monocytes from the bone marrow into the peripheral blood compartment would be $(10.5 \times 10^5)/32 = 3.3 \times 10^4$ cells per hr.

Promonocytes being multiplicative cells with a generation time of 19.5 hr, one promonocyte would give rise to $2 \times 1/19.5 = 0.1$ monocyte per hr. The production of 3.3×10^4 monocytes per hr would then require 3.3×10^5 promonocytes. Since the population of mononuclear phagocytes of the bone marrow consists of 15.9% promonocytes and 84.1% monocytes (2), the calculated total number of monocytes in the bone marrow would be 1.8×10^6 cells. Actually, we found that one femur contains 1.2×10^7 nucleated cells of which 1.2% are monocytes. Since the bone marrow in two femurs accounts for 11.8% of the total bone marrow mass (8), the total number of monocytes in the bone marrow of the mouse must be 2.4×10^6 cells, which agrees reasonably well with the calculated value. On the basis of the average of the calculated and observed values, the size of the monocyte pool of the bone marrow is about two times greater than the peripheral blood monocyte pool.

Kinetics of Promonocytes and Monocytes in the Bone Marrow Compartment.— We may now turn to the transit of cells through the promonocyte and monocyte pools of the bone marrow. If we assume one division in the promonocyte pool, the influx of cells from the pool of precursor cells into the promonocyte pool would be half the efflux from the latter pool. Since the hourly production of monocytes is 3.3×10^4 cells, the influx into the promonocyte pool will amount to 1.7×10^4 cells per hr. The relative influx of newly formed cells into the bone marrow monocyte pool can be calculated from the rise of the labeling index of bone marrow monocytes between 2 and 24 hr after labeling, which amounts to 32%, i.e., 1.5% per hour. Since a model population of 1000 bone marrow mononuclear phagocytes contains about 850 monocytes, the entry of new monocytes will amount to 12.8 cells per hr. Roughly similar results are obtained when these calculations are applied to the 150 promonocytes of the model population. As the promonocytes are multiplicative cells with a generation time of 19.5 hr, $150/19.5 \times 2 = 15.4$ cells per hr will leave the promonocyte pool and enter the bone marrow monocyte pool; this agrees with the above calculation of the influx. Since the monocyte pool contains 850 cells, the calculated value of the turnover time of the monocytes in the bone marrow compartment is $850/15.4 = 55.2$ hr. If the bone marrow monocytes were homogeneous and left the bone marrow according to a pipeline pattern, there

would be a delay of 55.2 hr before labeled monocytes appeared in the peripheral blood. However, in the present and earlier experiments (1), labeled monocytes appeared in the peripheral blood during the first hours after labeling.

During the first 18 hr after labeling, per hour twice as many newly formed monocytes appear in the bone marrow compartment as in the peripheral blood, but in the next 6 hr the release of monocytes per hour into the peripheral blood is about 3 times greater than the increase of labeled monocytes in the bone marrow (see Fig. 5). This suggests that kinetically the bone marrow monocytes are not homogeneous. Conceivably, one fraction of the bone marrow monocytes is fed into the peripheral blood rapidly, whereas another fraction arrives there after some delay.

SUMMARY

The mononuclear phagocytes of the bone marrow can be classified into two cell types, promonocytes and monocytes. The present study was performed to establish whether the promonocytes are the progenitors of the monocytes and to determine the kinetic characteristics of the promonocytes and monocytes in the bone marrow compartment.

Both in vitro labeling studies with thymidine-^{3}H and determination of the relative amount of DNA in the nuclei of individual cells showed that under normal steady-state conditions the promonocytes are proliferating cells and the monocytes, nondividing cells.

In vivo labeling studies provided further evidence that the promonocytes are the progenitor cells of the monocytes. During the first 24 hr after labeling, the promonocytes showed a constant high level of labeling (about 70%). The mean grain count of these cells decreased with time.

The labeling index of the monocytes of the bone marrow increased during the first 24 hr after in vivo labeling, but during the same period the mean grain counts remained almost constant, with values amounting to about half those of the promonocytes during the first 6 hr of the experiment.

The data concerning the labeling indices and the percentage distribution ratio of the promonocytes and monocytes in the bone marrow, and the labeling indices of the peripheral blood monocytes are used to construct a model population. The results lead to the conclusions that the promonocytes are multiplicative cells and that both daughter cells arising from the division of a promonocyte are monocytes.

The DNA-synthesis time found for the promonocytes is 13.6 hr. From this value, the average generation time was computed to be 19.5 hr.

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