Mitotic Indices of Human Bone Marrow Cells. I. Number and Cytologic Distribution of Mitoses

By Sven-Age Killmann, Eugene P. Cronkite, T. M. Fliedner and Victor P. Bond

The objectives of the present papers are threefold:

In part I, data on the mitotic indices of specific cell types (specific mitotic index) in normal human bone marrow will be presented.

In part II, restrictions in the use of mitotic index for computation of cytologic time parameters which have not been appreciated in previous work in this field will be pointed out. Failure to observe these restrictions may lead to erroneous estimates of cytologic time parameters.

In part III, the observed specific mitotic indices will be applied in computations of time parameters of hemopoietic cell proliferation in accordance with these restrictions.

Methods and Materials

Normal bone marrows were obtained from healthy males between the ages of 29 and 45. Bone marrow aspirations and squash preparations were done as described previously. Mitotic figures from earliest recognizable prophase to telophase (i.e., including nuclei with "twin" appearance and no nuclear membrane, lying close together) were counted in Feulgen-stained squash preparations because, with panchromatic stains of ordinary bone marrow smears, the mitotic index is underestimated, as pointed out by Japa. At least five slides from the same aspirate were counted to avoid errors due to possible local differences in proliferative activity. With the squash method it is not possible to assign mitotic figures to their specific cell line. Therefore, ordinary smears from the same aspirates were made and stained with Giemsa. In these preparations an appropriate number of mitotic as well as interphase cells were classified by one observer with respect to cell line and maturation level. The overall mitotic index and the cytologic distribution of mitotic and interphase cells were used to compute the specific mitotic index, i.e., the fraction of a particular cell type in mitosis at any time, of various hemopoietic cells. The specific mitotic index, e.g., of myelocytes, is computed from

\[
\text{mitotic figures/1000 cells} \times \frac{\text{myelocyte mitoses/100 marrow cell mitoses}}{\text{myelocytes/1000 marrow cells}}
\]

Both the morphology and the modal distribution of nuclear sizes of bone marrow cells in interphase suggest that there may be four dividing cell classes in the red cell series, and possibly the same number exists in the neutrophil series. Since cells in mitosis can not be classified in respect to their nuclear size or structure, classification rests on cytoplasmic staining characteristics and cell size. In previously reported data on thymidine labeling, erythroid and neutrophil precursors were classified according to nuclear size. In this way the proliferating compartments in either cell line could be broken down into four compartments.

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This research was supported by the U. S. Atomic Energy Commission.

Submitted Dec. 12, 1961; accepted for publication Feb. 12, 1962.

*From squash preparations.

†From Giemsa stained marrow smears.
Table 1.—Counts of 1000 Nucleated Cells in Separate Marrow Particles from the Same Aspiration from Five Healthy Males—Aspirations Done Between 1 P.M. and 8 A.M.

<table>
<thead>
<tr>
<th>Individual</th>
<th>Time*</th>
<th>Slides Counted</th>
<th>Mitotic Figures/1000 Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>TF</td>
<td>1:20 p.m.</td>
<td>5</td>
<td>7.0</td>
</tr>
<tr>
<td>TF</td>
<td>3:00 p.m.</td>
<td>6</td>
<td>6.5</td>
</tr>
<tr>
<td>TF</td>
<td>7:00 p.m.</td>
<td>5</td>
<td>10.8</td>
</tr>
<tr>
<td>TF</td>
<td>10:45 p.m.</td>
<td>5</td>
<td>13.4</td>
</tr>
<tr>
<td>TF</td>
<td>5:45 a.m.</td>
<td>5</td>
<td>7.4</td>
</tr>
<tr>
<td>EPC</td>
<td>5:00 p.m.</td>
<td>5</td>
<td>8.2</td>
</tr>
<tr>
<td>EPC</td>
<td>10:00 p.m.</td>
<td>5</td>
<td>6.0</td>
</tr>
<tr>
<td>EPC</td>
<td>10:45 p.m.</td>
<td>5</td>
<td>5.8</td>
</tr>
<tr>
<td>EPC</td>
<td>11:00 p.m.</td>
<td>5</td>
<td>11.8</td>
</tr>
<tr>
<td>VPB</td>
<td>10:20 p.m.</td>
<td>6</td>
<td>10.5</td>
</tr>
<tr>
<td>LF</td>
<td>12:45 a.m.</td>
<td>5</td>
<td>7.8</td>
</tr>
<tr>
<td>JB</td>
<td>8:00 a.m.</td>
<td>5</td>
<td>8.4</td>
</tr>
</tbody>
</table>

\[ \bar{x} = 8.63 \text{ SD } \pm 2.44 \]

*In TF, all marrow aspirations were done during one 24-hour period; in EPC, on different days.

†At 10 a.m., the mitotic index was 8.8.

With the morphologic criteria available for this study, these compartments could only be broken down into three subcompartments each (see Table 3).

**RESULTS**

1. **Diurnal Variation of Bone Marrow Mitotic Index**

   Previous work in this laboratory has shown that in bone marrow samples aspirated between 10 a.m. and 1 p.m., the overall mitotic index (i.e., the number of mitotic figures per 1,000 nucleated cells irrespective of cell type and degree of maturity) with the method employed is 9.0 ± 1.0. From animal work it is known that the mitotic activity in various tissues may show considerable diurnal variations. Therefore, bone marrow aspirations were done at different times of the day and the over-all mitotic index determined. The results are tabulated in Table 1. In one individual (T. F.), in whom all aspirations were done within one 24 hour period, the mitotic activity appears to be highest in the late evening hours. This trend, however, was not discernible in the rest of the observations. It is important to note that the mean (8.6) of these around the clock observations is very close to the mean of the mitotic indices in bone marrows obtained between 10 a.m. and 1 p.m. (9.0). For the estimates of bone marrow time parameters to be made in a following paper, it appears permissible, therefore, to pool our previous and present data on the mitotic index of normal bone marrow (Table 2).

2. **Ratio Between Erythroid and Myeloid Mitoses**

   The ratio between red and white cell precursor mitoses was determined from direct smears of bone marrow particles after staining with Giemsa. Twenty-five or 50 mitoses were counted in each of two different smears from 12 bone marrow samples aspirated at different times of the day from seven
Table 2.—Mitotic Indices (Mitotic Figures/1000 Nucleated Cells) Determined from Counts of Not Less Than 5000 Cells in 21 Different Bone Marrow Aspirations from Nine Healthy Males

<table>
<thead>
<tr>
<th>Individual</th>
<th>Number of Aspirations</th>
<th>Sum of Mitotic Indices in Each Individual</th>
<th>Mean of Mitotic Indices</th>
</tr>
</thead>
<tbody>
<tr>
<td>TF</td>
<td>7</td>
<td>61.4</td>
<td>8.77</td>
</tr>
<tr>
<td>EPC</td>
<td>5</td>
<td>40.5</td>
<td>8.1</td>
</tr>
<tr>
<td>VPB</td>
<td>2</td>
<td>18.9</td>
<td>9.45</td>
</tr>
<tr>
<td>LF</td>
<td>2</td>
<td>17.0</td>
<td>8.5</td>
</tr>
<tr>
<td>JB</td>
<td>1</td>
<td>8.4</td>
<td>(8.4)</td>
</tr>
<tr>
<td>VP</td>
<td>1</td>
<td>9.2</td>
<td>(9.2)</td>
</tr>
<tr>
<td>PH</td>
<td>1</td>
<td>9.0</td>
<td>(9.0)</td>
</tr>
<tr>
<td>GRS</td>
<td>1</td>
<td>10.3</td>
<td>(10.3)</td>
</tr>
<tr>
<td>SAK</td>
<td>1</td>
<td>8.0</td>
<td>(8.0)</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>Weighted Mean 8.70 ± 1.88 Mean 8.86 ± 0.73</td>
<td>Mean 8.86 ± 0.73</td>
</tr>
</tbody>
</table>

healthy males. Among 700 mitoses thus counted, 69.4 ± 5.74 per cent (SD) were mitoses in erythroid precursors, 28.2 ± 5.18 per cent (SD) were mitoses in myeloblasts, promyelocytes and neutrophil myelocytes, 1.9 ± .96 per cent (SD) were mitoses in eosinophil myelocytes, and 0.5 per cent were mitoses in other cells. A comparison of these percentages in bone marrows aspirated between 10 a.m. and 1 p.m. and during the rest of the day showed no significant differences.

3. Mitoses in Different Stages of Maturation

Two smears from each of 11 bone marrow aspirates from six healthy males were examined. In each slide, 25 or 50 cells in mitosis were classified according to cell type and maturity. A total of 650 mitotic cells were evaluated; the results are shown in table 3.

**DISCUSSION**

The Problem of Diurnal Variation of the Mitotic Index

In a previous report some estimates of turnover times in normal human bone marrow were made based on mitotic indices in bone marrow samples drawn between 10 a.m. and 1 p.m.¹ It was realized, however, that the mitotic index of the marrow need not remain constant throughout the day and that the figures used were not necessarily representative of the mitotic activity during a 24-hour period. In the present study, the observations in the one individual (T. F.) in whom six marrow samples were obtained during one 24-hour period would suggest such a variation with a peak during the evening hours; however, no such tendency can be found in the rest of the data. In order to establish or rule out diurnal changes of bone marrow mitotic activity in human beings, it will probably be necessary to do serial bone marrow aspirations within a 24- or 48-hour period under highly standardized conditions. The main reason for studying the mitotic index at different times of the day was, however, to ascertain whether the figure of 8.97 mitoses per
Table 3.—Distribution of 650 Mitotic Cells from Normal Bone Marrows According to Cell Type and Stage of Maturation

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Number of Mitoses</th>
<th>Relative Frequency of Mitoses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proerythroblast</td>
<td>31</td>
<td>1</td>
</tr>
<tr>
<td>Basophilic normoblast</td>
<td>127</td>
<td>4.1</td>
</tr>
<tr>
<td>Polychromatic normoblast</td>
<td>257</td>
<td>294</td>
</tr>
<tr>
<td>&quot;Orthochromatic&quot; normoblast*</td>
<td>37</td>
<td>9.5</td>
</tr>
<tr>
<td>Erythroid precursors, total</td>
<td>452</td>
<td></td>
</tr>
<tr>
<td>Myeloblast</td>
<td>18</td>
<td>1</td>
</tr>
<tr>
<td>Promyelocyte</td>
<td>36</td>
<td>2</td>
</tr>
<tr>
<td>Neut. myelocyte</td>
<td>128</td>
<td>7.1</td>
</tr>
<tr>
<td>Neutrophil precursors, total</td>
<td>182</td>
<td></td>
</tr>
<tr>
<td>Eos. myelocytes</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Lymphoid cell</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Reticular cells</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

*These mitotic cells have the size of polychromatic normoblasts.

1,000 nucleated cells (10 a.m.–1 p.m. samples) arrived at in a previous study was representative of the 24-hour mitotic activity. The weighted mean of all mitotic indices in the 1 p.m.–8 a.m. marrow samples was $8.63 \pm 2.44$ or $8.77 \pm 1.11$ if expressed as the mean of the mean mitotic indices of the five individuals studied in this time interval. If mitotic time is constant throughout the day, mitotic indices in marrows drawn between 10 a.m.–1 p.m. may therefore be regarded as representative of the marrow activity throughout the day. For the estimates to be made in the following, we have chosen to pool all our data as indicated in table 2 and use the figure of 8.86 mitoses per 1,000 nucleated cells as the normal average value.

Mitotic Activity in Erythropoiesis and Granulocytopoiesis

If in ordinary bone marrow smears there is no preferential loss of erythroid or myeloid mitoses, then the relative contributions of erythroid and neutrophil precursors to the overall mitotic index obtained from the squash preparations can be calculated from the ratio of erythroid to neutrophil precursor mitoses in bone marrow smears. Among 700 mitoses in ordinary bone marrow smears (same aspirates as used for mitotic index), 69.4 ± 5.74 per cent were found in the erythroid series and 28.2 ± 5.18 per cent in neutrophil precursors. These figures differ considerably from the data of Japa but are in agreement with the report by Dacie and White (67 of 100 mitoses counted were in erythroid precursors) and what can be calculated from the data of Videbaek (of 1,056 mitoses observed, 75.0 per cent were erythroid and 25.0 per cent myeloid). On the average, therefore, of the 8.86 mitoses/1,000 nucleated cells in the normal bone marrow, 69.4 per cent or 6.15 mitoses/1,000 are erythroid mitoses and 28.2 per cent or 2.50 mitoses/1,000 are neutrophil precursor mitoses.

Distribution of Mitoses on Different Maturation Levels

The relative frequencies of mitoses in cells at different maturation levels are listed in table 3. Apart from difficulties in cytologic identification of very
immature mitotic cells (myeloblasts), classification of mitotic cells is possible. Myeloblast mitoses were defined as mitotic cells about 15 μ in diameter with a pale blue cytoplasm without granules. Because of the inherent morphologic difficulties, it is possible that in some instances mitoses of other primitive cells have erroneously been classified as myeloblast mitoses. No mitoses were seen in small "orthochromatic" normoblasts; mitoses were infrequently encountered in large (i.e., size of polychromatic normoblasts) "orthochromatic" normoblasts. In normal bone marrow there are considerable discrepancies between the nuclear and cytoplasmic differentiation of erythroid precursors, a fact which in part explains the wide variations in classifications of these cells, and in the normal values reported for different classes of nucleated red cells. We consider these mitoses in large "orthochromatic" normoblasts to be mitoses in late polychromatic normoblasts with advanced hemoglobinization, a view which is supported by the fact that typical small "orthochromatic" normoblasts do not show any primary labeling with tritiated thymidine and hence do not synthesize DNA. To assure comparability of the counts, interphase "orthochromatic" normoblasts with nuclear and cytoplasmic dimensions of the polychromatic normoblast were also classified as polychromatic cells.

In table 3, the largest numbers of mitoses are contributed by the most mature cells capable of dividing, polychromatic normoblasts giving rise to about two-thirds of all erythroid mitoses and myelocyte mitoses representing more than two-thirds of all myeloid mitoses. If mitotic time does not vary with maturation level, the relative frequencies of mitoses (table 3) suggest that the erythroid series contains five consecutive divisions and the neutrophil series four (or five) consecutive divisions. The observed ratio of proerythroblast mitoses to other erythroid mitoses is 1: (4.1 + 9.5) = 1:13.6, and the observed ratio of myeloblast mitoses to other neutrophile mitoses is 1:9.1. If the proerythroblast is the ordinary stem cell of the red cell series, one of the two cells which emerge from a proerythroblast mitosis will remain a proerythroblast, whereas its sister cell will be destined for differentiation and further multiplication. With mitotic time constant and an orderly scheme of proliferation, the following would be observed: for each proerythroblast mitosis there will be one mitosis in the first daughter generation, two in the second daughter generation, four in the third generation, etc., so that the ratio of proerythroblast mitoses to mitoses in successive generations will be 1:1:2:4:8. In other words, if the proerythroblast is followed by four mitotable cell generations, the ratio of proerythroblast mitoses to other erythroid mitoses will be 1: (1+2+4+8) = 1:15, which is close to the observed value. In this event, a red cell on an average goes through five consecutive divisions provided that the assumptions are correct. If the proerythroblast is not a stem cell, then both cells which result from its division will differentiate, and the ratio of proerythroblast mitoses to other erythroid mitoses will be 1: (2+4+8) = 1:14. This means four consecutive divisions in the recognizable erythroid precursor cells plus one division in an unrecognized stem cell which feeds cells into the erythroid precursor pool. Similar considerations suggest between four and five consecutive divisions in the neutrophile series. If mitotic time increases with maturation, the number of consecutive divisions may be
Table 4.—Mean Bone Marrow Differential Count of 500 Cells in Each of Six of the Individuals Studied, Relative Compartment Sizes, and Specific Mitotic Indices

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Number Pr. 1000 Nucleated Cells in Bone Marrow Particle Smear</th>
<th>Relative Compartment Size</th>
<th>Relative Frequency of Mitoses</th>
<th>Number of Cells in Mitosis pr. 1000 Nucleated Cells</th>
<th>Specific Mitotic Index (fraction in mitosis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloblast</td>
<td>10</td>
<td>1.00</td>
<td>1.0</td>
<td>0.249</td>
<td>0.0249</td>
</tr>
<tr>
<td>Promyelocyte</td>
<td>33.7</td>
<td>3.37</td>
<td>2.0</td>
<td>0.498</td>
<td>0.0148</td>
</tr>
<tr>
<td>Myelocyte</td>
<td>163.0</td>
<td>16.30</td>
<td>7.1</td>
<td>1.778</td>
<td>0.0109</td>
</tr>
<tr>
<td>Non-dividing marrow granulocytes*</td>
<td>361.4</td>
<td>36.14</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Proerythroblast</td>
<td>16.7</td>
<td>1.00</td>
<td>1.0</td>
<td>0.422</td>
<td>0.0253</td>
</tr>
<tr>
<td>Basophilic normoblast</td>
<td>35.0</td>
<td>2.10</td>
<td>4.1</td>
<td>1.729</td>
<td>0.0494</td>
</tr>
<tr>
<td>Polychromatic normoblast</td>
<td>70.7</td>
<td>4.23</td>
<td>9.5</td>
<td>3.998</td>
<td>0.0565</td>
</tr>
<tr>
<td>Orthochromatic normoblast</td>
<td>102.0</td>
<td>6.11</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Metamyelocytes to marrow segmented neutrophils.

†From table 3.

‡Calculated from relative frequency of mitoses on different maturation levels and 2.50 total neutrophile and 6.15 total erythroid mitoses pr. 1000 nucleated cells.

§Specific mitotic index = fraction of specific cell type in mitosis at any time.

Bone marrow cells not pertinent to the present study were excluded from the table.

less. In table 4, the specific mitotic index (the fraction of one particular cell type in mitosis at any time) is tabulated. The highest specific mitotic index is found in the most immature cells of the neutrophil series and in the most mature cells of the dividing erythroid series. The mitotic index is much greater in the erythroid than in the neutrophil series, the specific mitotic index in the former being up to five times greater than in the latter.

The present mitotic indices are higher than previously reported values. The most likely explanation is that with the squash technic used for establishing the overall mitotic index of the bone marrow, more mitotic figures are recognized than in conventional bone marrow smears. The specific mitotic indices then are high because they were computed from this overall mitotic index and the cytologic distribution of mitoses in Giemsa stained marrow smears. Most earlier reports of hemopoietic mitotic indices have dealt with gross mitotic indices of erythropoietic and granulocytopoietic cells as a whole. A notable exception is the work of Schwarz who counted the mitotic index of proerythroblasts separately and found a higher mitotic index in proerythroblasts and a lower index in the more mature red cell precursors than in the present study. The probable reason for this is differences in cytologic classification of mitotic and interphase cells. Schwarz thus found the ratio of proerythroblast mitoses to other normoblast mitoses to be approximately 1:2. From the present data, this ratio is 1:13.5; however, if mitoses in proerythroblasts and basophilic normoblasts were combined in one group, the ratio would be 1:1.86.
From the present data, some estimates of bone marrow time parameters are possible. The use and restrictions of mitotic indices for such computations will be examined in the following paper. In a third paper, the actual computations will be presented and the results compared with estimates obtained by other methods.

**Summary**

1. The diurnal variation of mitotic index of human bone marrow has been studied. A diurnal variation has not been demonstrated but remains subject for further study.

2. Data on the distribution of bone marrow mitoses on cell lines and maturation stages are presented. From this and the gross mitotic index of the bone marrow, the mitotic indices of various cell types are computed.

**Summary in Interlingua**

1. Esseva studiate le variation diurne del indice mitotic de human medulla ossee. Le question de un possibile variation diurne non esseva resolvite sed remane aperte pro studios additional.

2. Es presentate datos super le distribution de mitoses in le medulla ossee secundo typos de cellula e stadios de maturation. A base de iste datos e del grossier indice mitotic del medulla ossee, le indices mitotic es computate pro le varie typos de cellula.

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parameters in proliferating cell sys-
tems with particular reference to
serially connected multiplicative com-
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Erythro- and granulocytopoietic time
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