Mitotic Indices of Human Bone Marrow Cells.
III. Duration of Some Phases of Erythrocytic and Granulocytic Proliferation Computed from Mitotic Indices

By S.-A. Killmann, E. P. Cronkite, T. M. Fliedner, and V. P. Bond

DATA on the mitotic indices of human bone marrow cells were reported in the first paper of this series, and theoretical considerations on the applicability and limitations of the index in determining kinetic parameters were discussed. In the present paper an attempt is made to compute time parameters of normal bone marrow cell proliferation from these data and principles.

It is recognized that there is perhaps a diurnal variation in the mitotic index. However, as discussed this possibility does not significantly influence the utilization of these data. In order to extract information on the temporal parameters of proliferation of bone marrow cells from mitotic indices, a number of assumptions are necessary. All recognized assumptions will be stated explicitly, with the double purpose of demonstrating the limitations of the conclusions which can be drawn from these and similar data and which illustrate the complexity of the subject. As demonstrated, exact information about the actual structure of a proliferating compartment is a prerequisite for precise interpretation of mitotic indices. Therefore, a brief discussion of some aspects of hemopoietic cell proliferation is necessary before the computations are presented.

Both erythro- and granulocytopoiesis are initiated by a self-perpetuating stem cell compartment. During the maturation that follows, the cells go through a series of cytologic transitions and consecutive divisions, and finally they reach a stage (orthochromatic normoblast, metamyelocyte) where mitosis ceases or is a rarity under normal conditions. After a period of further maturation and storage, the cells (although perhaps not all cells) are released from the marrow as mature, functioning units.

The morphological identity of the hemopoietic stem cell(s) is not clear. For the purpose of the present computations it was assumed that the proerythroblast is the usual stem cell for erythropoiesis. In any event, it is generally agreed that the proerythroblast is a normal stage of erythrocytic development; a small inflow of cells (small as compared to recognizable


*This also applies to interpretation of autoradiographic studies following labeling of DNA with tritiated thymidine or other pyrimidines where changes in labeling index are followed.

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red cell production) from an earlier stem cell compartment* would not materially change calculations. The choice of the myeloblast as the stem cell of the neutrophil series is more debatable, since it has not been established that the myeloblast normally participates in neutropoiesis. The promyelocyte is accepted as a granulocytopoietic precursor and is believed by some to be the usual stem cell for neutropoiesis. In principle, it is an important question whether there is an inflow of cells into the promyelocyte compartment from the myeloblast compartment (or some other compartment). However, the quantitative effect on neutrophil kinetics will not be very significant as long as this possible inflow of cells is small compared to cell production in the recognizable granulocytic precursor compartments. In fact, cell contribution from stem cell compartments is now believed to be small in the “steady state” equilibrium for all cell production, although under severe stress this may become a major source of new cell production, particularly in erythropoiesis.

More important for our estimates is the problem whether all cells of a certain morphological cell class in the bone marrow belong to the same cell generation, or whether one cell class may comprise more than one cell generation. The first possibility implies that the morphologic change with cell division is irreversible and so pronounced that the cells of the daughter generation can be clearly distinguished from the cells of the mother generation. For this the term “heteromorphogenic division” is used. If cells of the mother and daughter generations resemble each other sufficiently to make a clearcut cytologic distinction between them impossible, the division is said to be “homomorphogenic.”

Heteromorphogenic cell divisions have been seen by direct observation of metamyelocytes arising from dividing myelocytes. Also, labeled metamyelocytes and orthochromatic normoblasts appear within a few hours after the corresponding dividing precursor cells have been labeled with H3-thymidine. Since some time elapses from completion of DNA-synthesis to mitosis it is probable that the last division in the erythrocytic and neutrophilic series approximates being heteromorphogenic.

While it seems clear that mitoses of the most mature dividing cells may approximate being heteromorphogenic, the nature of earlier mitoses is unclear. Apparently in favor of heteromorphogenic divisions are the karyometric studies by Weicker which demonstrated that the nuclear diameters of erythropoietic cells show a multimodal distribution. It is claimed that each frequency maximum represents one cell generation. However, the distribution of cell diameters around the modal values overlap so that a considerable number of cells can not with confidence be assigned to a particular generation. Granulocytopoietic cells are not as suitable for karyometric

*In earlier papers (Conkite, et al., 8,11,12,19) the hypothetical common stem cell compartment has been termed the primitive progenitor pool (PPP). However, arguments and data of Loutit strongly imply if they do not actually prove a common stem cell compartment.
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classification because of the more irregular nuclear shape. Nevertheless, Weicker stated that small myelocytes and metamyelocytes have the same nuclear volume \( K^{1/2} \) and concluded that the small myelocyte is a non-dividing cell. This is inconsistent with the fact that about 25 per cent of small myelocytes incorporate \( H^3 \)-thymidine and thus are synthesizing DNA in preparation for mitosis. Normal metamyelocytes do not synthesize DNA. This indicates that cells with identical nuclear volumes (small myelocytes-metamyelocytes) do not necessarily belong to the same cell generation. Also data on the relative frequency of mitoses at different maturation levels, interpreted in the preceding paper, suggest that the mitotic rate of the various multiplicative hemopoietic cell compartments exceeds cell entry rate even when allowing for the slight increase in mitotic time with maturation observed in time lapse photographic studies of human bone marrow cultures. This means that on an average a cell which enters the compartment will divide, and one or both daughter cells will also divide within the same compartment. In other words, the cytologic compartments contain more than one cell generation. Obviously, this is merely an expression of the difficulties in exact cell identification, the result of which is that cells of different generations may be assigned to the same cytologic class.

In conclusion, some marrow mitoses are definitely heteromorphogenic. There are good reasons to believe, however, that not all divisions follow this pattern. As demonstrated the position of mitosis in a compartment has a distinct influence on the computation of compartment transit time (i.e., the average time from entrance of a cell into a cytologic compartment until it or its progeny leaves the compartment) from mitotic index. Since the position of mitosis in the various bone marrow compartments is uncertain, the computations must be restricted to maximum and minimum compartment transit times as discussed in the preceding paper.

Estimate of Mitotic Time

In the following estimates of mitotic time and DNA-synthesis time, two assumptions were made:

1. The myeloblast and proerythroblast are the stem cells of their respective cell series, i.e., there is no or very little inflow of cells into the myeloblast and proerythroblast compartments during "steady state" conditions. If there is an inflow, mitotic time and DNA-synthesis time are longer than estimated.

2. A possible death function (cell abortion) in the proliferating compartments is negligible. A large death function would mean that cell production is higher than estimated from the transit time of non-dividing cell compartments, and mitotic time and DNA-synthesis time would be shorter than estimated here.

The observed transit time of non-dividing marrow neutrophils in man—

*Alternatively, each compartment might contain a self-sustaining subcompartment with a proliferation scheme like stem cells, which would also explain the discrepancy between cell entry rate and mitotic rate.
that is, the time from cell entry into the metamyelocyte compartment to segmented granulocyte exit to the blood—is about 3½ days. In a population of 1,000 bone marrow cells, 361.4 are non-dividing neutrophilic cells (table 1). This number of cells is replaced in 3½ days (84 hours). Hence the number of cells entering the non-dividing neutrophil compartment per hour is 361.4/84 = 4.3. In the steady state and assuming no death function in the proliferating compartments, this number is equal to the total number of mitoses per hour in the proliferating pool. At any time, 1,000 bone marrow cells contain 2.50 neutrophil precursor mitoses (mitotic pool). Per hour, 4.3 cells enter and leave this mitotic pool of 2.50 cells. Therefore, the mitotic time of neutrophil precursors (= transit time of mitotic pool) is 2.50/4.3 = 0.58 hours.

The upper limit for the transit time of orthochromatic normoblasts is estimated at 19 hours. Using the figures of table 1, 102.0 orthochromatic normoblasts are replaced in 19 hours. Hence, the inflow per hour is 5.38 cells. At any time, 6.15 red cell precursor mitoses are seen in this marrow population sample. Therefore, the upper limit of mitotic time of red cell precursors is 6.15/5.38 = 1.14 hours. Since transit time of orthochromatic normoblasts was an upper limit, the mitotic time derived from it is also an upper limit. As will be discussed later, erythroid mitotic time is probably only about 0.75 hours. It can not be determined from the present data whether mitotic time varies with maturation stage, but the work of Rondanelli et al. indicates that such variations are small.

Estimate of DNA-Synthesis Time

The weighted mean of DNA-synthesis time (tDNA) can be estimated in the same manner and with the same limitations as mitotic time. When all mitotable neutrophil cells are pooled, about 50 per cent of them will "flash label" with H3-thymidine in vivo. With 206 mitotable neutrophils per 1,000 bone marrow cells (table 1), about 103 neutrophil precursors will be labeled. Hence, tDNA = 103/4.3 = 24 hours. Of all mitotable red cell precursors about 62 per cent will "flash" label. Hence, tDNA for red cell precursors is 122.4* × 0.62/5.38 = 14.1 hours. The estimated tDNA in red cell precursors is an upper limit estimate since it is derived from an upper limit of orthochromatic transit time. As discussed later, 9 hours is probably a better estimate.

Estimate of Compartment Transit Times

These estimates are based on the following assumptions:

(1) That the estimate of mitotic time is correct. It will be remembered that the estimate of mitotic time in the erythroid series is a maximum value. Therefore, as discussed later, the transit times of erythrocytic compartments should probably be reduced by about one third.

*1,000 bone marrow cells contain 122.4 mitotable erythroid cells (table 1).
Table 1.—Mean Bone Marrow Differential Count of 500 Cells in Each of 6 Individuals Studied, Relative Compartment Sizes, and Specific Mitotic Indices (from Ref. 1)

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Number Per 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 per 1000 Nucleated Cells</th>
<th>Specific Mitotic Index (fraction in mitosis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloblast</td>
<td>10.0</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.
†Calculated from relative frequency of mitoses on different maturation levels and 2.50 total neutrophil and 6.15 total erythroid mitoses per 1000 nucleated cells.

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

Definition of cell types: 29 myeloblast = Neutrophiloblast; 29 promyelocyte = neutrophiler Promyelocyt I + II; 29 (myelocyte = halbreifer + reifer neutrophiler Myelocyte; 29 proerythroblast = Pronormoblast + Makronormoblast; 29 basophilic normoblast = basophiler Normoblast; 29 polychromatic normoblast = polychromatischer Normoblast; 29 orthochromatic normoblast = oxypilhe Normoblasten. 29

(2) That mitotic time does not vary with the level of cell maturation. Although this may not be strictly true, variations appear to be small. 10

(3) That myeloblasts and proerythroblasts are the stem cells of their respective cell lines. Any inflow into these compartments from a preceding unrecognized precursor pool would change the time estimates somewhat, particularly in the early compartments. However, such a hypothetical inflow of cells would mean that mitotic time is longer than estimated (because mitotic time was found by relating the total inflow into non-dividing compartments to the number of mitoses in the recognized precursor pool) which to some degree would counterbalance the effect of inflow on computation of transit times.

(4) The cells proceed through the cell line in an orderly fashion, i.e., that each cell in the process of maturation and division goes through the same sequence of events as every other cell of the same type.

(5) That a possible death function in the proliferating pool is negligible. However, as pointed out previously, a high death function would mean that mitotic time is shorter than estimated and hence is to some degree com-
As discussed in detail previously, the compartment transit time (CTT) of a compartment is determined by the cell entry rate ($K_{in}$) from the preceding compartment and the mitotic rate ($K_{M}$) within the compartment. In the steady state, the cell exit rate ($K_{out}$) from the compartment will equal the sum of $K_{in}$ and $K_{M}$. The following estimates are based on the mitotic times computed above and on mitotic indices and relative compartment sizes of human bone marrow (table 1). The number of cells in a compartment is designated $N$.

a. Myeloblasts. Assuming that the myeloblast is the stem cell of the neutrophil series its CTT can be computed from $\frac{N}{K_{M}}$. Among 1,000 myeloblasts, 24.9 are observed in mitosis at any time. With a mitotic time of 0.58 hours, the number of myeloblast mitoses per hour per 1,000 myeloblasts is $24.9/0.58 = 43.0$, and $\text{CTT} = 1,000/43.0 = 23.3$ hours.

b. Promyelocytes. The size of the promyelocyte compartment which corresponds to a myeloblast compartment of 1,000 cells is $3.37 \times 1,000 = 3370$ cells. Maximum CTT is computed from $\frac{N}{K_{in}}$. From the myeloblast compartment, 43.0 cells enter the promyelocyte compartment per hour. Hence, maximum $\text{CTT} = 3370/43.0 = 78.3$ hours.

Minimum CTT can be computed from $\frac{N}{K_{in} + K_{M}}$. Among 3370 promyelocytes, $14.8 \times 3.37 = 50.0$ are observed in mitosis at any time. With mitotic time = 0.58 hours, the number of promyelocyte mitoses per hour per 3370 promyelocytes will be $50.0/0.58 = 86.2$. Minimum $\text{CTT} = \frac{3370}{(43.0 + 86.2)} = 26.0$ hours.

c. Other cells. In the same manner, minimum and maximum compartment transit times of other proliferating bone marrow compartments can be estimated. The results are given in table 2. By adding the transit times of table 2 one gets the upper and lower limit of the total transit time through the cell line. The time from the proerythroblast stage to the last normoblast division could vary from 2-5 days. From the myeloblast to the last myelocyte division, the elapsed time could vary from 3½ to 9½ days.

Comparison of Present Estimates with Previous Estimates

The estimates of mitotic time are in fair agreement with other reported values for mitotic time in mammals. As pointed out above, the mitotic time of erythropoiesis is an upper limit since it was derived from an upper limit estimate of transit time through the non-dividing compartment. Since this

*The time from when a cell enters a cytologically defined compartment until it or its progeny leaves the compartment.
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Table 2.—Estimated Compartment Transit Times of Hemopoietic Compartments (Hours)

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Minimum CTT (from N/K&lt;sub&gt;out&lt;/sub&gt;)</th>
<th>Maximum CTT (from N/K&lt;sub&gt;M&lt;/sub&gt;)</th>
<th>CTT (from N/K&lt;sub&gt;M&lt;/sub&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proerythroblasts*</td>
<td>—</td>
<td>45 (30)</td>
<td></td>
</tr>
<tr>
<td>Basophilic normoblasts*</td>
<td>18.6 (12.4)</td>
<td>95.0 (63.5)</td>
<td>--</td>
</tr>
<tr>
<td>Polychromatric normoblasts*</td>
<td>13.1 (8.8)</td>
<td>37.5 (25.0)</td>
<td>--</td>
</tr>
<tr>
<td>Orthochromatric normoblasts</td>
<td>19†</td>
<td>19†</td>
<td>--</td>
</tr>
<tr>
<td>Myeloblasts</td>
<td>—</td>
<td>23.3</td>
<td>--</td>
</tr>
<tr>
<td>Promyelocytes</td>
<td>26.0</td>
<td>78.3</td>
<td>--</td>
</tr>
<tr>
<td>Neutrophil myelocytes</td>
<td>37.4</td>
<td>126</td>
<td>--</td>
</tr>
<tr>
<td>Non-dividing marrow neutrophils†</td>
<td></td>
<td>84§</td>
<td></td>
</tr>
</tbody>
</table>

With the estimates of mitotic time used (1.14 hours in erythropoiesis, 0.58 hours in granulocytopoiesis) and the assumptions stated in the text, the figures in the table indicate the extremes between which the true compartment transit times must lie. The computations are based on the data in table 1 and are explained in the text.

*It should be noted that the estimate of mitotic time in erythropoiesis is an upper limit value; if mitotic time is shorter the estimates of transit times must be lowered proportionately. As discussed in the text, estimates of erythroid transit times should probably be lowered to about 2/3 of the values tabulated. These “best estimates” are shown in parenthesis.

†From Ref. 6.
‡Metamyelocytes to marrow segmented neutrophils.
§From Ref. 6, 9, 12.
N = total number of cells in compartment.
K<sub>out</sub> = cell exit rate.
K<sub>in</sub> = cell entry rate.
K<sub>M</sub> = mitotic rate.

For definition of cell types, see table 1.

work was completed, Odartchenko et al. measured mitotic time directly by observation of the progression of label through the mitotic window of late (polychromatic) normoblasts. Their observations and those of Bond et al. strongly suggest that mitotic time for late normoblasts is less than one hour and close to 0.6 hours.

The estimate of DNA-synthesis time is higher than estimates for human tissue culture cells, and mouse tissues. In the red cell series, DNA may be brought in accordance with these other estimates by assuming that the estimate of orthochromatric normoblast transit time is high by a factor of 2. The estimated DNA in granulocytopoiesis (24 hours) is rather long but is in agreement with a H<sup>3</sup>-thymidine labeling index of about 0.5 and a generation time of neutrophil precursors, derived from decrease in grain count, of about 2 days.

The average generation time of human bone marrow cells was estimated by Fliedner et al. and Cronkite et al. on the basis of mitotic indices. The
figures for erythroid and neutrophilic mitotic indices differed somewhat from those used in this paper. They arrived at 11–33 hours for red cell precursors and 25–75 hours for neutrophil precursors. These figures refer to all mitotable cell types as a whole and depend on the assumption—unrecognized at that time—that the last division in both series is heteromorphogenic. The range of the estimates is explained from mitotic time which was assumed to be between 0.5 and 1.5 hours. They also estimated the total transit time of cells from the earliest precursor cell to the terminal division by multiplying the computed average generation time by the number of consecutive divisions within the cell series. This type of computation is applicable only if generation time is constant in all consecutive generative cycles. From data on DFP in vivo labeling of neutrophils, Mauer et al. consider the average generation time of neutrophil precursors to be 3 days. H²-thymidine data indicate a neutrophil generation time of about 2 days. The present data suggest a weighted average generation time of 24 hours or less in mitotable nucleated red cells and about 48 hours in neutrophil precursors provided that the mitotable pools are not diluted with non-dividing cells, i.e., that the terminal mitosis is heteromorphogenic.

Calculations of the “turnover time” of red and white cell precursors have been presented by Patt. Turnover time was defined as “time required to make a number of cells equal to the number in a series.” As discussed, the conceptual content of the term “turnover time” varies with the type of cell renewal system to which it is applied. Like Leblond in his studies of the renewal of intestinal epithelium, Patt computed the “turnover time” of bone marrow cells from the mitotic time divided by mitotic index. Mitotic index was defined as mitoses in cell line/mitotable + non-mitotable cells. When applied to a simple cell renewal system consisting of a stem cell compartment feeding directly into a non-dividing compartment, this expression will yield the sum of the average stem cell generation time and the average time spent in the non-dividing compartment. When this expression is applied to more complicated systems as the bone marrow (and perhaps the gut) with consecutive divisions prior to the non-dividing compartment, the resulting “turnover time” will equal the weighted average generation time of the proliferating pool plus the average time spent in the non-dividing compartment. Patt calculated the “turnover time” of nucleated erythrocytic cells to be 23 hours and that of neutrophils to be 75 hours, but pointed out that these times were less than the time of development from early progenitor cells to release from the marrow. These “turnover times” were subdivided into “average time spent in proliferating class” (5.75 hours for red cell precursors, 12.5 hours for neutrophils) and “maturation time in post mitotic stage” (17.25 and 62.5 hours respectively for erythroid and neutrophil cells). The latter term can not be misinterpreted and is synonymous with “CTT of non-dividing compartment” in our terminology (< 19 hours for orthochromatic normoblasts, about 84 hours for non-dividing marrow neutrophils). The term “average time spent in proliferating class” is less clear. The way
it was computed indicates that it means the weighted average generation time of the proliferating class, provided that this is not diluted with non-dividing cells. Patt used other data for mitotic index and assumed a mitotic time of 0.5 hours. This explains why his estimate is lower than ours. It should be noted that the average time it takes a cell to go from an early stage of development to its (or strictly: its progeny cell’s) last division is longer than the weighted average generation time. For this reason we prefer the term “weighted average generation time” to “average time spent in proliferating class.”

Preliminary estimates of compartment transit times based on the present data have been reported previously. These estimates differ from the values given in table 1 for two reasons. First, mitotic time was assumed to be one hour and equal in erythroid and granulopoiesis. Second, the transit times were computed from N/K and therefore were minimum values. The present compartment transit time of myeloblasts (1 day) is close to Cronkite et al.’s estimate of generation time of myeloblasts (1.3 days) based on decrease in radioactivity after labeling with H3-thymidine. This may suggest that myeloblasts have a heteromorphogenic division (fig. 4; 1 in Ref. 2) or—since practically 100 per cent of myeloblasts label with H3-thymidine—a heteromorphogenic division with apparent dedifferentiation (fig. 4; 3 in Ref. 2). However, with the uncertainties involved in the estimates this must remain speculation for the time being. The transit time of proerythroblasts is estimated to be 45 hours whereas their generation time from H3-thymidine data appears to be 1.1–1.2 days. This discrepancy could be explained by a homomorphogenic proerythroblast division (fig. 4; 2 in Ref. 2). However, in that case it would be difficult to understand that practically 100 per cent of proerythroblasts label with H3-thymidine, and therefore it is more likely that the difference is due to an overestimate of mitotic time and hence of transit times in the red cell series. This view is supported by the work of Rondanelli et al. who found no appreciable difference between mitotic time in red and white cell precursors whereas the present estimate of erythroid mitotic time is almost twice that of neutrophil mitotic time.

Table 3.—Estimates of Erythropoietic and Granulocytopoietic Cell Mass in Standard Man

<table>
<thead>
<tr>
<th>Author</th>
<th>Nucleated Red Cell Precursors</th>
<th>Neutrophils and Precursors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osgood</td>
<td>8.6</td>
<td>25.7*</td>
</tr>
<tr>
<td>Patt</td>
<td>3.4</td>
<td>8.3</td>
</tr>
<tr>
<td>Suit</td>
<td>4.6</td>
<td>—</td>
</tr>
<tr>
<td>Donohue et al.</td>
<td>5.0</td>
<td>11.4</td>
</tr>
<tr>
<td>Mauer et al.</td>
<td>—</td>
<td>12.5</td>
</tr>
<tr>
<td>Present report</td>
<td>5.2 (3.5)</td>
<td>13.1 (8.8)</td>
</tr>
</tbody>
</table>

*Segmented forms excluded.
†These values are based on an upper limit estimate of erythroid mitotic time and probably are high by a factor of about 0.3. The corrected figures (“best estimates”) are given in parenthesis.
Estimate of Total Marrow Cellularity

From the data which have been used to estimate compartment transit times, it is also possible to compute total marrow cellularity and the blood transit time of neutrophil granulocytes. Comparisons with existing measurements of granulocytes per hour can be computed from

\[
\frac{\text{frequency of neutrophil mitoses}}{\text{frequency of erythroid mitoses}} \times \frac{\text{erythroid mitotic time}}{\text{neutrophil mitotic time}} \times \frac{\text{RBC produced per hour}}{6.15} = 6.95 \times 10^9
\]

neutrophils produced per hour in a 70 kg. man.

Recently, Athens et al.\(^2\) have demonstrated the existence of two granulocyte pools of about equal size in the blood: a circulating pool and a marginal pool. However, equilibrium between these two pools is rapid and complete, and kinetically they may be considered as one pool. According to their data the total blood granulocyte pool (N) in standard man (70 kg.) contains 46 \(\times\) 10\(^9\) neutrophil granulocytes.

Their data suggest that the loss from the blood is random. A random loss has now been shown autoradiographically by Fliedner et al.\(^2\) In the “steady state” the loss from the blood must equal the input into the blood. Therefore with a random loss, the rate of loss is

\[
\frac{\text{dN}}{\text{dt}} = \lambda N
\]

\(\lambda\) is the fractional replacement per hour. With an input of 6.95 \(\times\) 10\(^9\)/hour

\[
\lambda = \frac{6.95 \times 10^9}{46 \times 10^9} = 0.15
\]

The average time in the blood (\(\frac{1}{\lambda}\)) is 6.7 hours and the half-time is 4.6 hours. If the erythroid mitotic time is shortened to 0.7 hours then \(\lambda = 0.094\); average time is 10.7 hours, and the half-time is 7.4 hours.

The computation of the half-time and average time in the blood for granulocytes is based on the assumption that the mitotic rates are correct. The production of red cells is well established. The computation of neutrophil production is then insensitive to the mitotic times providing that both are proportionately longer or shorter than the true average mitotic times. However, if the erythroid mitotic time is overestimated disproportionately in respect to neutrophil mitotic time, the neutrophil production will be overestimated. If erythroid mitotic time is underestimated disproportionately, the neutrophil average time in the blood will be underestimated because the neutrophil production is underestimated and vice versa. From H\(^3\)-thymidine data Bond et al.\(^6\) computed 2.3 days as the upper limit of the turnover time (pipeline not random loss from blood) of blood granulocytes. They pointed out that the actual time could be much shorter if unlabeled and labeled cells entered the circulation at the same time. From curves showing the progression with time of labeled cells through the postmitotic neutrophil
compartments of the bone marrow,\textsuperscript{9,12} it appears that the labeled cell population does not move as a coherent body. It is likely, therefore, that considerable mixing with unlabeled cells has occurred at the time when labeled granulocytes reach the point of release from the marrow into the blood. This is supported by the studies with DFP\textsuperscript{22} labeling of granulocytes by Mauer et al.\textsuperscript{20} which showed a half-time of circulation blood granulocytes of 6.4 hours and a "turnover time" (average time in blood) of 9.4 hours. The difference between this value and the present estimate could be rationalized by assuming that a minor fraction of bone marrow segmented neutrophils never enters the blood. Teleologically it would appear appropriate for the organism to maintain a marrow reserve of fully differentiated neutrophils for immediate emergencies which—if not utilized—ultimately die in their organ of origin. Although this remains a possibility, the discrepancy between the present estimate and the one based on DFP\textsuperscript{22} data may also be explained in another way. If erythroid mitotic time were only two-thirds of the value used in the computation above (i.e., 0.76 hours rather than 1.14 hours), then the estimate of granulocyte production would be lowered by one-third and consequently the blood transit time would be in very good agreement with the DFP\textsuperscript{22} measurements. This is one more reason to suspect that erythroid mitotic time has been overestimated. A priori it was known that this estimate was an upper limit because it was derived from an upper limit estimate of orthochromatic normoblast compartment transit time. Reducing erythroid mitotic time by about one-third would bring the present estimate of granulocyte blood transit time close to the value derived from DFP\textsuperscript{22} data and would yield a compartment transit time of proerythroblasts close to proerythroblast generation time deduced from H\textsuperscript{3}-thymidine data. Moreover, erythroid and neutrophil mitotic time would be almost identical which fits with the direct measurements of mitotic time in short term bone marrow cultures of human bone marrow by Rondanelli et al.\textsuperscript{10} If erythroid mitotic time is about 0.75 hours rather than 1.14 hours, all red cell precursor transit times computed in this paper, Bond et al.'s\textsuperscript{6} estimate of orthochromatic normoblast transit time, and the present estimate of erythroid DNA-synthesis time and generation time and of total marrow cellularity must be reduced by about one-third.

Since the completion and acceptance of this paper for publication, studies of Patt et al.\textsuperscript{30} and Lala et al.\textsuperscript{31} have become available. Patt et al.\textsuperscript{30} estimated the proliferation rate of granulocytopoiesis in the dog based on labeling studies with tritiated thymidine and concluded that there is probably a significant "death" at the myelocyte level. They do not eliminate a "death function" at more and less mature levels of maturation.

The estimation of production rates in our work assumes no death of cells in the marrow proliferative compartment. We assume a net growth of one cell for each mitosis. Production rate measured in this manner conforms to the time estimates of loss from the blood by Mauer et al.\textsuperscript{20} and Athens et

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and would not support the ideas of Patt et al. on a "myelocyte sink." However, it is clear that the problem of a myelocyte sink can only be answered to our satisfaction if all studies on the flow rates in the compartments of the proliferative pool and the replacement in the metamyelocyte pool are performed in the same individuals with H\textsuperscript{3}-thymidine labeling and mitotic index simultaneously. Until this is done the subject remains open. Lala et al. studied the mitotic index of human beings and found a significantly higher mitotic index than did we. However, their technic was different.

**SUMMARY**

1. Weighted average mitotic time of human bone marrow cells is estimated. Mitotic time is about 0.75 hours in red cell precursors and about 0.58 hours in neutrophil precursors.

2. Estimated weighted average DNA-synthesis time is about 9 hours in red cell precursors and about 24 hours in neutrophil precursors.

3. Upper and lower limits for compartment transit times of proerythroblasts, basophilic normoblasts, polychromatic normoblasts, myeloblasts, promyelocytes and myelocytes are presented (table 2).

4. Total mass of nucleated red cell precursors is estimated at $3.5 \times 10^9$/kg, and total mass of neutrophils and their precursors in the marrow at $8.8 \times 10^9$/kg.

**SUMMARIO IN INTERLINGUA**

1. Es estimate le ponderate valor medie del tempore mitotic de cellulas de human medulla ossee. Le tempore mitotic es circa 0.75 horas in precursores de erythrocytos e circa 0.58 horas in precursores de neutrophilos.

2. Es estimate que le ponderate valor medie del tempore de synthese de acido desoxyribonucleic es circa 9 horas in precursores de erythrocytos e circa 24 horas in precursores de neutrophilos.

3. Le limites superior e inferior pro le tempore de transito compartamental de proerythroblastos, notmoblastos basophile, normoblastos polychrome, myeloblastos, promyelocytos, e myelocytos es presentate in forma tabular.

4. Le massa total de nucleate precursores de erythrocytos es estimatemente $3.5 \times 10^9$ per kg de peso. Le massa total de neutrophilos e lor precursores in le medulla es estimatemente $8.8 \times 10^9$ per kg.

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Mitotic Indices of Human Bone Marrow Cells. III. Duration of Some Phases of Erythrocytic and Granulocytic Proliferation Computed from Mitotic Indices

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