The Size and Transit Time of Nondividing Subpool of Precursor Cells in Acute Leukemia

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The labeling index of acute leukemia precursor cells has been found to be much smaller than that of normal precursor cells. Available data indicate that the estimated generation time of leukemic cells is not long enough to account for the much smaller labeling index of leukemic cells as compared to that of normal cells, assuming that the DNA synthesis time of leukemic cells is equal to that of normal cells. Consequently, the existence of a nondividing fraction among precursor cells has been postulated in acute leukemia. To further substantiate the postulation of a nondividing fraction it is necessary, however, to exclude the possibility that the DNA synthesis time of leukemic cells might be very much shorter than that of normal cells. The knowledge of DNA synthesis time of normal cells has been relatively well established; that of leukemic cells, however, is hardly available. The purpose of this paper is to substantiate further the postulation of the nondividing subpool of acute leukemia precursor pool and to present an in vitro experimental estimation of its size and transit time.

Prolonged-Labeling Model and Computational Formulas

The experimental model in the present study involves a prolonged labeling with tritiated thymidine (3H-TDR). The labeling index is followed as a function of labeling time. The model assumes (1) a steady state, (2) a rigorously orderly progression along the cell cycle, (3) a self-maintained cytologic compartment or pool terminated by heteromorphogenic division, and (4) continuous availability of 3H-TDR for DNA labeling. (See "Discussion." ) The symbols \( t_c, t_s, t_{c1}, t_{c2}, \) and \( t_m \) were used to designate the durations of cell cycle, DNA synthesis phase \( (S) \), presynthesis rest phase \( (G_1) \), postsynthesis rest phase \( (G_2) \), and mitotic phase \( (M) \), respectively.

Homogeneous Population

If all cells can divide, the labeling index as a function of time will be a linear line with:

\[
\beta = \frac{1}{t_c} \quad \ldots \ldots \quad (1)
\]
intercept \( a \), \quad a = \frac{t_a}{t_c} \quad \ldots \ldots (2) \]

\[
\frac{(2)}{(1)}, \quad t = \frac{a}{\beta_1} \quad \ldots \ldots (3)
\]

All cells will be labeled at and after time \( t = t_c \).

**Heterogeneous Population**

The population is considered to consist of two subpools, dividing and nondividing ones, cytologically indistinguishable from each other. The latter is fed solely by the former. The cell exit from the nondividing subpool is on a cohort basis with all cells spending equal time, the “nondividing subpool transit time” (NSTT), in this subpool. The nondividing cells cannot synthesize DNA and hence will not be initially-labeling on flash labeling. (See “Discussion.”)

As the labeled dividing cells divide, statistically 50 per cent of their labeled daughter cells will remain as dividing cells with the other 50 per cent moving out of the dividing subpool. This movement is either into the nondividing subpool without cytologic changes or out of the cytologic pool under consideration due to cell differentiation. Let \( x \) be the fractional size of the nondividing subpool; \( 1-x \), that of the dividing one; \( y \), the fractional movement out of the dividing subpool into the nondividing one, and \( 1-y \), the other due to cell differentiation. The labeling index as a function of time will consist of consecutive linear segments with initial labeling index (intercept, \( a \)) and successive slopes (\( \beta_1, \beta_2, \) and \( \beta_3 \)) as given below:

the first slope over the period equal to \( t_{x_1} + t_m \), \( \beta_1 = \frac{1-x}{t_c} \) \quad \ldots \ldots (1)

the second slope over the period between \( t_{x_1} + t_m \) and \( t_{x_2} + t_m \), \( \beta_2 = \beta_1 (1 + y) \) \ldots \ldots (2)

the third slope over the period after \( t_{x_2} + t_m \), \( \beta_3 = y\beta_1 \) \ldots \ldots (3)

the initial labeling index,

\[
\alpha = \frac{t_a(1-x)}{t_c} \quad \ldots \ldots (4)
\]

\[
\text{Eq}(2)/(1) \quad t = \frac{a}{\beta_1} \quad \ldots \ldots (5)
\]

From (2)

\[
y = \frac{\beta_2}{\beta_1} - 1 \quad \ldots \ldots (6)
\]

At time \( t = t_c \), when all dividing cells would have been labeled, the labeling index \( (I_L) \) can be shown to be

\[
I_L = (1-x) + \frac{y(1-x)t_{a1}}{t_c} \quad \ldots \ldots (7)
\]

The nondividing subpool transit time is given by \( \text{NSTT} = \frac{x}{y\beta_1} \) \ldots \ldots (8)

The model presupposes a NSTT longer than \( t_{x1} \) of the dividing cells because available evidences\(^{3,4}\) and the present study indicate that the labeling index of leukemic cells is much less than unity even when the labeling time is prolonged to encompass one generation time.

**Materials and Methods**

Four cases were studied. The two leukemic patients were virgin cases without any treatment prior to study. Case 1 (Patient P. B.) was a 37-year-old female with rheumatic
heart disease and normal hemopoiesis. Case 2 (Patient J. H.) was a 38-year-old female with functional gastrointestinal disorder and normal hemopoiesis. Case 3 (Patient J. B.) was a 56-year-old male having acute myeloid leukemia with palpable spleen tip, total white blood cell count of 4700/mm³ and sternal marrow showing 95 per cent myeloblasts or promyelocytes. Case 4 (Patient H. W.) was a 69-year-old male having subacute myeloid leukemia with total white blood cell count of 113,000/mm³ and sternal marrow markedly replaced by myeloblasts (10 per cent) and promyelocytes (40 per cent).

Methods

Sternal marrow was collected in panheprin. Red cells were removed by dextran sedimentation. A final nucleated cell suspension with approximately 4000/mm³ was made in pooled fresh-frozen human serum, Basal Medium of Eagle, L-glutamine, and minute quantities of antibiotics, and containing 0.3–0.5 μc. of ³H-TDR (6.7 C/mM) per ml. of suspension. Labeling characteristics were determined for the “myeloid precursor pool” which includes myeloblasts, promyelocytes, and myelocytes, but not metamyelocytes or later forms. The labeling index was determined at 90 per cent confidence level; the mean grain count was based on evaluation of 50–120 cells; the per cent of labeled mitosis and average grain count per mitosis were based on evaluation of 10–80 myeloid mitoses. The average background over nondividing cells (bands and neutrophils) was estimated at 0.2. Cells of 4 or more grains were considered labeled. Labeling characteristics were measured at 1, 5, 13, 19, 25, and in Cases 3 and 4 also at 37 hours after the beginning of incubation. Cells were washed three times in Hanks’ solution; smears were made and coated with NTB-3 emulsion, exposed for 7 days (Cases 3 and 4) or 14 days (Cases 1 and 2), developed in D-19, fixed in F-5 and stained with Harleco Giemsa at pH 5.75.18

Results and Calculations

The change of marrow population in the short-term culture (see “Methods”) was studied in two other cases (Patients M. O. and B. B.) both with nonhematologic conditions and normal hemopoiesis. Results are shown in Tables 1 and 2. Serious deviation from steady state did not occur for myeloid precursor pool as a whole. Mitotable nucleated red cells decreased rapidly with time as was also observed by Lajtha.19

Results of labeling characteristics in Cases 1–4 are shown in Figures 1–4.

Case 1 (Fig. 1)

The labeling index increased almost linearly with time. Linear regression was applied to the first five points (0, 4, 8, 12, and 18 hours) to obtain the slope (0.026/hour) and the intercept (0.35). From Eq(1), t₁ was 13 hours. \( t_{\text{c2}} + \frac{1}{2}t_m = 6.8 \) hours. \( t_2 \) was obtained in two ways: (a) \( t_2 = t_1/a = 37 \text{ hours} \). (b) \( t_2 = t_1 + t_c = 24 + 13 = 37 \text{ hours} \).

Case 2 (Fig. 2)

The labeling index versus time was appreciably deviated from linearity. The initial rate of increase in labeling index over the first four hours was taken for the slope. \( \beta = \frac{1}{4} (0.49--0.38) = 0.0275/\text{hour} \). \( \alpha = 0.38 \). From Eq (1), \( t_c = 14 \text{ hours} \). \( t_2 = t_2/a = 37 \text{ hours} \). \( t_{\text{c2}} + \frac{1}{2}t_m = 5.2 \text{ hours} \).

Case 3 (Fig. 3)

To determine the first and second slopes, the \( t_{\text{c2}} + \frac{1}{2}t_m \) of 8.6 hours, as estimated by the 50 per cent labeling intercept, was taken as an approximation of
Fig. 1.—Case 1: P. B. with normal hemopoiesis. Labeling index, average grain count per labeled, per cent mitosis labeled, and average grain count per mitosis, for myeloid precursors, as functions of labeling time. $^3$H-TDR, 0.05 μc. per ml. cell suspension; exposure, 14 days.

Fig. 2.—Case 2: J. H. with normal hemopoiesis. Labeling index, average grain count per labeled, per cent mitosis labeled, and average grain count per mitosis, for myeloid precursors, as functions of labeling time. $^3$H-TDR, 0.03 μc. per ml. cell suspension; exposure 14 days.
Fig. 3.—Case 3: J. B. with acute myeloid leukemia. Labeling index, average grain count per labeled, per cent mitosis labeled, and average grain count per mitosis, for myeloid precursors, as functions of labeling time. $^{3}$H-TDR, 0.05 μc. per ml. cell suspension; exposure, 7 days.

$G_2 + M$ time which corresponds to the first inflection of the function of labeling index. Linear regression for the first three points (0, 4, and 8 hour) gave $\beta_1 = 0.0033$/hour and $\alpha = 0.054$. From Eq(3), $t_s$ was 16 hours. Linear regression for the four points—8, 12, 18, and 24 hours—gave $\beta_2 = 0.0044$. From Eq(4), $y = 0.34$ (the fractional movement into the nondividing subpool).

The function of labeling index leveled off abruptly after 24 hours. Hence $t^* - t_s$ was about 24 hours and $t_s = (t^* - t_*) + t_s = 40$ hours approximately.

By Eq(2), $0.054 = 16(1-x)/40$, and the size of nondividing subpool, $x$, was about 0.87 or 87 per cent. Labeling index at 24 hours was 0.16. The $t_{el}$ of 15.4 hours was estimated by $(t_1 - t_*) - (t_2 + \frac{1}{2}t_m)$ approximately. Thus by

Eq(5), $0.16 = 1-x + \frac{1}{40}0.34(1-x) 15.4; x = 0.86$ or 86 per cent approximately.

By Eq(6), $\text{NSTT} = 0.86/(0.0033-0.34) = 768$ hours or 32 days.

Case 4 (Fig. 4)

Because of the considerable deviation from the model, the estimation of the size and transit time of the nondividing subpool was not possible. The initial slope of the function of labeling index over the first four hours was taken for $\beta_1$. The initial labeling index was $\alpha = 0.12$. $\beta_1 = (0.15-0.12)/4 = 0.0075$/hour. Thus by Eq(3), $t_s$ was about 16 hours.
DISCUSSION

Validity of Assumptions

The results of Tables 1 and 2 reflect the validity of steady state assumption. In real systems random fluctuation exists and one is in effect only estimating the average value for each kinetic parameter. The result of Table 1 suggests that the assumption of self-maintained myeloid precursor pool is justifiable. Heteromorphogenic terminal myeloid division was directly observed in vitro by Boll and Kuhn. Regardless of the type of terminal division, Eq(3), from which \( S \) times for Cases 3 and 4 were computed, holds valid.

In Cases 1 and 3, the per cent mitosis labeled eventually reached and stayed at 100 per cent; the average grain count per mitosis increased over what one might take roughly as an estimate of \( S \) time and reached a plateau. These mitotic data assure that cells were continuously entering \( S \) phase from \( G_1 \) phase and were adequately labeled at least during the early part of incubation. In Cases 2 and 4 their mitotic data indicate that inadequate availability of \( ^3 \)H-TDR later in culture was responsible for the deviation of the function of labeling index from the model. In Case 4, whose deviation was most pronounced, the per cent mitosis labeled fell to 68 per cent at 36 hours, indicating that later in culture some cells were not labeled even though they were in, or had gone through, the \( S \) phase.

Rapid degradation of thymidine into thymine and dihydrothymine by leukocyte thymidine phosphorylase and a TPNH-dependent system in vitro was demonstrated by Marsh and Perry and subsequently confirmed by Cooper.
PRECURSOR CELLS IN ACUTE LEUKEMIA

Table 1.—Total Nucleated Cell Count* and Absolute Differential Cell Counts† per mm.³ for Marrow Cells in Short-term Culture on Patient M. O.

<table>
<thead>
<tr>
<th>Hours</th>
<th>0</th>
<th>1</th>
<th>3</th>
<th>6</th>
<th>12</th>
<th>18</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total nucleated cell</td>
<td>2813</td>
<td>2525</td>
<td>2663</td>
<td>2775</td>
<td>2868</td>
<td>2613</td>
<td>2906</td>
</tr>
<tr>
<td>Total myeloid precursor</td>
<td>375</td>
<td>380</td>
<td>332</td>
<td>400</td>
<td>367</td>
<td>403</td>
<td>370</td>
</tr>
<tr>
<td>Myeloblast</td>
<td>21</td>
<td>19</td>
<td>16</td>
<td>15</td>
<td>10</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>Promyelocyte</td>
<td>38</td>
<td>32</td>
<td>25</td>
<td>33</td>
<td>28</td>
<td>23</td>
<td>25</td>
</tr>
<tr>
<td>Myelocyte</td>
<td>306</td>
<td>314</td>
<td>285</td>
<td>356</td>
<td>339</td>
<td>365</td>
<td>332</td>
</tr>
<tr>
<td>Metamyelocyte</td>
<td>354</td>
<td>319</td>
<td>317</td>
<td>361</td>
<td>376</td>
<td>329</td>
<td>393</td>
</tr>
<tr>
<td>Band</td>
<td>405</td>
<td>402</td>
<td>516</td>
<td>345</td>
<td>425</td>
<td>392</td>
<td>463</td>
</tr>
<tr>
<td>Segment</td>
<td>725</td>
<td>786</td>
<td>806</td>
<td>929</td>
<td>956</td>
<td>793</td>
<td>1027</td>
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<td>Total nucleated red cell</td>
<td>420</td>
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<td>225</td>
<td>200</td>
<td>205</td>
<td>165</td>
<td>150</td>
</tr>
<tr>
<td>Pronormoblast</td>
<td>12</td>
<td>8</td>
<td>7</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Basophilic normoblast</td>
<td>42</td>
<td>39</td>
<td>25</td>
<td>24</td>
<td>20</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>Polychromatic normoblast</td>
<td>191</td>
<td>136</td>
<td>133</td>
<td>110</td>
<td>114</td>
<td>84</td>
<td>70</td>
</tr>
<tr>
<td>Orthochromatic normoblast</td>
<td>156</td>
<td>110</td>
<td>67</td>
<td>56</td>
<td>72</td>
<td>71</td>
<td>74</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>489</td>
<td>372</td>
<td>394</td>
<td>409</td>
<td>445</td>
<td>454</td>
<td>454</td>
</tr>
</tbody>
</table>

*Coefficient of variation is 5 per cent.
†Calculated from total nucleated cell count and differential count of about 10,000 cells on each slide.

Table 2.—Total Nucleated Cell Count* and Absolute Counts* of Most Immature Cells per mm.³ for Marrow Cells in Short-term Culture on Patient B. B.

<table>
<thead>
<tr>
<th>Hours</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>12</th>
<th>18</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total nucleated cell</td>
<td>2570</td>
<td>2581</td>
<td>2662</td>
<td>2594</td>
<td>2537</td>
<td>2490</td>
</tr>
<tr>
<td>Myeloblast</td>
<td>27</td>
<td>22</td>
<td>20</td>
<td>17</td>
<td>16</td>
<td>13</td>
</tr>
<tr>
<td>Promyelocyte</td>
<td>20</td>
<td>19</td>
<td>28</td>
<td>18</td>
<td>14</td>
<td>20</td>
</tr>
<tr>
<td>Pronormoblast</td>
<td>10</td>
<td>6.2</td>
<td>2.8</td>
<td>1.7</td>
<td>0.8</td>
<td>0.2</td>
</tr>
<tr>
<td>Basophilic normoblast</td>
<td>25</td>
<td>19</td>
<td>14</td>
<td>13</td>
<td>5.6</td>
<td>2.2</td>
</tr>
</tbody>
</table>

*See Table 1.

and Milton. The variable decrease in the availability of ³H-TDR in our four cases is likely attributable to the unavoidable variation in the mechanical aspect of marrow processing with variable release of catabolic enzymes or factors into the supernatant. Another possible source of the irregularity is the fact that the fresh-frozen human serum used in Cases 1 and 3 was from one batch and that used in Cases 2 and 4 was from another. The high specific activity of ³H-TDR used and the low nucleated cell count of the incubation mixture left undisturbed until termination of labeling are likely the factors responsible for the relative success of prolonged labeling in the present study.

Average Grain Count per Labeled Cell

The average grain count per labeled cell increased rapidly over the first four hours in every case. At 4 hours it was 1.6 to 2.6 times as much as that at zero hour. The peak time varied but occurred in every case at a time after substantial number of labeled mitosis had appeared. The peak time does not need to correspond to t₂ + tₜ. In Case 3, whose t₂ + tₜ was estimated as about 24 hours, one would expect to see a second rising wave of grain count after 24 hours. In both Cases 3 and 4 only a suggestion of leveling-off after 24 hours was
observed. Apparently mitotic halving of grains had outweighed continuous but weak labeling of cells in S phase due to decreasing availability of $^3$H-TDR with time, while the weak labeling still gave grains above labeling threshold.

**DNA Synthesis Time and Generation Time**

Since decreasing availability of $^3$H-TDR with time tends to underestimate the slopes of the functions of labeling index, the estimated S times may represent overestimates particularly in Cases 2 and 4. Our results indicate, however, that the S time of myeloid precursors in acute myeloid leukemia is not much different from, and is no shorter than, that of normal myeloid precursors. This view is supported to some extent by the rough estimate of 20 hours given by Ota for the S time of blast cells in a case of acute myeloid leukemia. Thus calculations based on the data of labeling index at zero hour, S time, and C time of our four cases also give the size of nondividing subpool in our leukemic patients as about 86 per cent.

**Nondividing Subpool of Precursor Cells in Acute Leukemia**

There have been suggestions or evidences for the existence of nondividing fraction among leukemic cells.2,3,6-7

**Hypothesis of Existing Nondividing Fraction Being Maintained by the Dividing Fraction—Analytical Review of Literature.**

Cytogenetic Data: Current cytogenetic data25-26 suggest that the dividing cells during active phase of acute leukemia consist entirely or predominantly of mutant leukemic cell line and lend support to the assumption that the nondividing subpool, which obviously consists of leukemic cell line, is fed solely or mainly by the dividing subpool.

Circulating Leukemic Cells as Nondividing Cells—Flash Labeling Index Data and Feulgen-Cytophotometric Data: It has been repeatedly shown that the flash labeling index of blast cells in acute leukemia is remarkably lower in blood than in marrow.2,4,6 In addition, Feulgen-cytophotometric studies6,27 have shown that the great majority of circulating leukemic cells are presynthesis-resting population. It is, therefore, very tempting to conclude that the great majority of circulating blast cells belong to the nondividing fraction. Furthermore, since mitosis of leukemic cells in the blood is an extreme rarity,2 the observations2,5,6 of the gradual increase of per cent labeled cell in the blood eventually to about that in the marrow over the first two days following a flash in vivo labeling, imply that the circulating nondividing cells originate in the marrow or other production sites. It is also implied that the nondividing subpool in maintained at least mainly by the dividing subpool, since blast cells labeled at the production sites are released into the circulation where they are nondividing. The blood environment does not have inhibitory effect on the $^3$H-TDR uptake by either normal cells or marrow leukemic blasts.2 It seems, therefore, that the nondividing subpool is an end cell subpool rather than a "stem cell subpool" feeding into a "dividing multiplicative subpool."

Possibility of Initial-Labeling of Some Nondividing Leukemic Cells: In every case of Ota's series8 of acute leukemia, for which the grain count per labeled marrow blast as a function of time following in vivo flash labeling is given, the
grain count at a time when all initially-labeled blasts would have divided once as judged by the rough knowledge of $M + G_2 + S$ time in vivo, is about the same as or at least much greater than half of the initial grain count. Thus it appears that some nondividing leukemic cells may synthesize DNA and be initially-labeled. Release of initially-labeled nondividing cells from the marrow into the circulation might well account for the observation of Killmann et al.\textsuperscript{2} that “the most heavily labeled cells in the blood did not have a lower mean grain count than the most heavily labeled cells in the marrow.”

Overestimation of C Time by Grain-Halfing Methods: The estimates of C time obtained by various grain-halfing methods,\textsuperscript{2,6} which assume a homogeneous population, are remarkably longer than that obtained by labeled mitosis method,\textsuperscript{8} which is independent of the population homogeneity.\textsuperscript{28} The wide discrepancy is most readily explained by rejecting the assumption of homogeneous population made in grain-halfing methods. That the overestimating effect inherent otherwise in the grain-halfing method itself does not cause this much discrepancy in acute leukemia may be seen from the in vivo data\textsuperscript{8,29-30} on myeloid precursor cells in nonleukemic subjects.

The Function of Per Cent Labeled Cell Versus Time with Flash in Vivo Labeling: The initial increase in per cent labeled cell for later multiplicative compartments with in vivo flash labeling in normal hemopoiesis\textsuperscript{31-33} has been explained by the higher per cent labeled cell of the earlier compartment.\textsuperscript{32} Interestingly, in every case of acute leukemia for whom the per cent labeled marrow blast as a function of time is given in published papers,\textsuperscript{2,5-6} the per cent labeled blast also increases initially and, at a time when all initially-labeled mitotable blasts would have divided once, is significantly higher than (in some cases even twice as high as) its initial value at one hour. Although the previous explanation\textsuperscript{32} cannot apply, the paradox is readily explained by the hypothesis under discussion.

Chronic in Vivo Labeling—Failure of 100 Per Cent Labeling: The release of leukemic blasts from the marrow into the circulation appears randomized.\textsuperscript{2} If one assumes a random exit of nondividing cells from the production site, then theoretically with a chronic in vivo labeling, the function of labeling index at the production site is approximately a combination of linear and monomolecular functions for the period between $t_{c2} + t_m$ and $t_t - t_8$, and a monomolecular function for the period after $t_t - t_8$, approaching 100 per cent labeling at infinite time.\textsuperscript{34} It is, therefore, not surprising that multiple or chronic in vivo labeling in acute leukemia\textsuperscript{2,5,6} should have failed to achieve 100 per cent labeling within finite time up to 10 days.

Interpolation of in vitro Data to in vivo Situation. If the assumption that all nondividing cells are not initially-labeling is falsely made in the model, the initial labeling index in Eq(2) will be greater than $t_6(1-x)/t_c$ and also the first slope will be greater than $(1-x)/t_c$. The overall effect of this likely false assumption on the estimate of t, by Eq(3) in Cases 3 and 4 can not be predicted due to lack of precise knowledge regarding nondividing cells.

For a closed system in vitro it is probably safer to assume cell exit from nondividing subpool on a cohort basis than on a random basis. Regardless
of the nature of this exit, the 32-day estimate of nondividing subpool transit time in Case 3 can apply only to a closed in vitro system. There would be subdivisions of nondividing subpool in vivo. The transit time in the circulation seems relatively short. The transit time through the production site such as the marrow is likely very long as to overpopulate the production site and thus interfere with the normal hemopoiesis.

The estimate of 87 per cent for the size of nondividing subpool in Case 3 reflects the population heterogeneity of leukemic cells in the marrow. Since almost all leukemic cells in the blood are nondividing, the nondividing subpool size would be even greater than 87 per cent in Case 3 when the total leukemic cells in the body are concerned.

Summary and Conclusions

1. In order to elucidate the postulation and to quantitate the size and transit time of the nondividing subpool of precursor cells in acute leukemia, mathematical formulas based on a prolonged-labeling model were derived for the following kinetic parameters: (1) DNA synthesis time and generation time of dividing precursor cells in normal hemopoiesis and in acute leukemia. (2) size and transit time of the nondividing subpool of precursor cells in acute leukemia. The validity of the assumptions made in the model was discussed.

2. In vitro experimental estimation of the above kinetic parameters was done for the myeloid precursor pool in four cases: two with normal hemopoiesis; one, acute myeloid leukemia; one, subacute myeloid leukemia.

3. Variable decrease with time in availability of tritiated thymidine given initially in single dose to the in vitro system was observed and discussed.

4. DNA synthesis time and generation time of the dividing myeloid precursor cells in the two leukemic patients were not significantly different from, and were no shorter than, the corresponding values for normal myeloid precursor cells in the two nonleukemic patients. Thus it allows the prediction that as much as 86 per cent of the myeloid precursor cells in the marrow may be nondividing in acute myeloid leukemia.

5. In a case of acute myeloid leukemia about 87 per cent of the myeloid precursor cells in the marrow were estimated as nondividing with nondividing subpool transit time of about 32 days in closed in vitro system. The probable meanings of these in vitro estimates in vivo were discussed.

6. The result and analytical review of literature support the postulation of heterogeneous acute leukemia precursor cells with the nondividing fraction consisting of end cells being maintained by the dividing fraction. It is also suggested that a small fraction of nondividing cells may be capable of DNA synthesis and thus be initially-labeling. More definitive approaches to the problems await future investigation.

Summario in Interlingua

1. Con le objectivo de elucidar le postulation e de quantificar le dimension e le tempore de transito del nondivisible subpool de cellulas precursori in
leucemia acute, formulas mathematic basate in un modello a markage pro-
longate eseva derivate pro le sequente parametros kinetic: (1) tempore de
synthese e tempore de generation de acido deoxyribonucleic in cellulas
precursori in division in hematopoiese normal e in leucemia acute, e (2)
magni-
tude e tempore de transito del nondivisibile subpool de cellulas precursori
in leucemia acute. Le validitate del suppositiones postulate pro le modello es
commentate.

2. Un estimation experimental in vitro del supra-mentionate paramet-
ros kinetic eseva effectuate pro le pool de precursores myeloide in quatro casos:
du o con hematopoiese normal, un con leucemia myeloide acute, e un con
leucemia myeloide subacute.

3. Esseva observate e es commentate un variabile declino con le tempore in
le disponibilitate de thymidina a tritium administrate initialmente in un dose
unic al systema in vitro.

4. Le tempore del synthese e le tempore de generation de acido deoxyribo-
nucleic de cellulas precursori myeloide in division in le duo patientes leucemic
non eseva significatamente differente ab le correspondente valores pro nor-
mal cellulas precursori myeloide in le duo patientes nonleucemic. Assi il deveni
possible predicer que usque ad 86 pro cento del cellulas precursori myeloide
in le medulla non se divide in acute leucemia myeloide.

5. In un caso de acute leucemia myeloide, il eseva estimate que circa 87
pro cento del myeloide cellulas precursori in le medulla non se divideva e que
le tempore de transito in le subpool sin division eseva circa 32 in le claudite
systema in vitro. Le signification probable de iste estimationes pro processos
in vivo es commentate.

6. Le resultos e le revista analytic del litteratura supporta le postulation de
heterogenee cellulas precursori in leucemia acute con le fraction non in division
consistente de cellulas terminal mantenite per le fraction in division. Es etiam
suggestionate que un micro fraction de cellulas nondivisori es possibilemente
capace do synthetisar acido deoxyribonucleic e assi de markage initial. Plus
definitive methodos pro le solution del problemas va resultar de investigationes
futur.

APPENDIX

Statistical Technic Used

1. Normal Approximation of Hypergeometric Distribution
(a) Hypergeometric Distribution
N: total number of myeloid precursors
r: number of labeled myeloid precursors
s: number of myeloid precursors sampled
D: number of labeled myeloid precursors in the sample

\[
P(D = d) = \frac{\binom{r}{d} \binom{N - r}{s - d}}{\binom{N}{s}}
\]
Let I be the unbiased estimate of the true value of the labeling index.

\[ I = \frac{D}{s} \cdot \frac{r}{N} \]

Hence \( \text{Var}(I) = \frac{N - s}{N - 1} \cdot \frac{1}{s} \cdot \frac{1}{N} \).

Since \( N \) is far greater than \( s \),

\[ \text{Var}(I) \approx \frac{1}{s} \]

so

\[ \text{SD}(I) = \sqrt{\frac{1}{s}} \]

(b) Normal Approximation

For a confidence interval of \((I \pm d)\) at a confidence level of 90 per cent or more,

\[ d > 1.645 \cdot \text{SD}(I) \text{ or } d > 1.645 \cdot \sqrt{\frac{1}{s}} \]

and so

\[ s > \frac{1.645^2 \cdot (1 - \bar{r})}{d^2} \]

The sample size \( s \) of myeloid precursors in this study was guided by Eq. (7). The sample size was determined for \( d = 0.02 \) in Case 1 and Case 2 and for \( d = 0.01 \) in Case 3 and Case 4.

2. Linear Regression

Details of linear regression will not be given here since they are available in textbooks on statistics. The formulas for the slope and intercept are shown below:

\[ a = \frac{\sum Y_i - \beta \sum X_i}{n} \]
\[ \beta = \frac{\sum X_i Y_i - \sum X_i \sum Y_i}{n \sum X_i^2 - (\sum X_i)^2} \]

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