**Leukocyte Kinetics in Hematologic Disorders Studied by DNA-Phosphorus Labeling**

By Richard I. Walker, J. C. Herion, W. B. Herring and J. G. Palmer

The application of tracer methods to the study of leukocyte kinetics has led to improved understanding of the normal physiology of leukocyte production, movement, and destruction, but the considerable variation in estimates of leukocyte life span attests to the difficulty of interpretation of such studies. The use of deoxyribonucleic acid (DNA) labeling to study the kinetics of cell production is founded on evidence that DNA synthesis occurs only in cells preparing for division and that after final mitosis, no significant exchange of DNA constituents occurs. Labeling of DNA with inorganic ortho-radiophosphate ($^{32}$P) has been used by a number of investigators to study leukocyte kinetics.

After intravenous injection of $^{32}$P in normal individuals, label appears in the DNA of circulating leukocytes, reaching a maximum concentration several days following isotope administration. The general shape of the curve is quite similar in the various species studied, although time relations vary. It has been shown in rabbits and humans that lymphocyte labeling does not contribute significantly to the shape of the circulating leukocyte DNA-P specific activity curve. Since circulating leukocytes do not normally synthesize quantitatively significant amounts of DNA, the shape and height of the DNA labeling curve will be the resultant of duration and rate of DNA synthesis in marrow in relation to the concentration of label in the precursor pool and the subsequent movement of cells into and out of the blood. Striking qualitative differences between the curves found in normal and disease states have been reported. This report describes the results of such studies in hematologically normal humans and in patients with chronic leukemia, polycythemia vera and myelofibrosis.

**Materials and Methods**

These studies were done in human volunteers, each of whom received intravenously 2.0 µc. of $^{32}$P/Kg. of body weight. Diagnoses were established according to accepted clinical definition after a complete history, physical examination and the appropriate laboratory determinations. In all cases, the quantity and character of the formed elements in the peripheral blood were determined by routine clinical laboratory procedures. Peripheral blood smears and marrow smears and sections were examined in all cases by the

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authors. Red cell mass was determined by the isotope dilution method with Cr51-tagged cells; arterial oxygen saturation and other indicated studies were performed to establish the diagnosis of polycythemia vera. Blood urea nitrogen and serum phosphorus were determined to exclude renal failure and hyperphosphatemia in patients with any history of renal disease. Table 1 presents pertinent laboratory findings in the patients studied.

Blood was obtained at intervals after isotope administration, and leukocytes were isolated by dextran sedimentation, hemolysis of red cells remaining with the leukocytes, and removal of platelets by differential centrifugation. In the latter part of these studies, red cell hemolysis was effected by a 30-second exposure to 0.22 per cent saline2 rather than by gramicidin-lysolecithin. Since platelets contain no DNA,26 the number of washings and centrifugations was reduced to two, one before and one after hemolysis, when only DNA was to be examined. The DNA-P specific activity of samples so processed did not differ significantly from that of duplicate samples obtained by the more lengthy technic. DNA was obtained from the leukocytes by a modification of the Schmidt-Thannhauser procedure and the specific activity of DNA phosphorus was determined by relating radioactivity measured by scintillation counting to phosphorus content quantitated by the method of Berenblum and Chain. Details of these procedures have been previously published.19,27

RESULTS

Plasma clearance of inorganic P32 after intravenous administration to a patient with polycythemia vera is shown in figure 1. This curve resembles that found in a normal human by Perry et al.,17 and that reported by Lala et al.22 in patients with chronic granulocytic leukemia. A significant concentration of isotope is present for at least 5 days and no doubt much longer, since the rate of decline in activity is very slight at this time.

The results shown in the upper part of figure 2 were obtained from a man and a woman hospitalized for physiotherapy several months after a cerebrovascular accident and ligation of an intracranial arterial aneurysm respectively; both were hematologically normal when studied. These curves are similar in shape to those in normals reported by others.1,14,17 The concentration of label remains at a low level for 5–6 days and then increases rapidly during the next 2 days, reaching a maximum 8 days after injection of P32. The rate of decline in label concentration is more gradual than the rate of increase.

Curves with a configuration similar to those from normals were obtained in studies in two patients with untreated polycythemia vera (fig. 3). The apparent differences in slope are likely of no significance, representing chiefly the arbitrary way in which curves are drawn and the small size of the sample.

The low, flat curves of circulating leukocyte DNA-P specific activity (fig. 2, lower part) obtained in three patients with chronic lymphocytic leukemia are in striking contrast to the above. Two of these patients had received chemotherapy several months previously, but were in relapse and receiving no therapy at the time of study. The hump in the curve obtained from patient L.R. probably results from activity in granulocytes that represented up to 20 per cent of circulating cells during the period of study. Lymphocytes made up 92–99 per cent of circulating cells in the other two patients. Table 2 contains data from studies in patient L.R. after 2.0 and 20.0 μc. of P32/Kg. Circulating leukocyte DNA-P specific activity resulting from 20 μc. P32/Kg.
approximates 10 times the level reached after 2.0 μC/Kg. No significant change in the total or differential counts of circulating cells occurred during either study. That high levels of specific activity do not occur in circulating lymphocytes immediately after isotope administration is shown by the extremely low values in various fractions of these cells obtained from another patient with chronic lymphocytic leukemia during the first 3 1/4 hours after he received 2.0 μc. of P³²/Kg. (table 3).

Curves quite different from all the preceding ones are found in studies in patients with chronic granulocytic leukemia (fig. 4). These patients were either previously untreated or in relapse on no therapy; and in each the number of the various circulating leukocytes remained relatively constant during the period of study. After P³² injection, increase in label concentration in circulating leukocyte DNA-P is progressive. This could obscure the presence of a population of cells with relatively normal kinetic behavior. The maximum label concentration achieved is considerably lower than that seen in normals, although the time of its occurrence is about the same. A secondary increase in rate of label accretion is suggested in all curves, but is most obvious in the curve for H.G. Observations made beyond day 10 in A.M. (days 17, 24, 31, 38), in A.A. (day 17), and T.R. (days 11, 12) show a gradually decreasing concentration of label, approaching that found in plasma. The maximum in each patient occurs during the first 10 days.

Studies were repeated in four patients after a course of therapy with busulfan. Patient H.G., off therapy for 3 weeks and receiving no therapy during the study, and patient T.R., who continued to receive 2 mg. of busulfan daily, were both in complete remission; their total and differential blood leukocyte counts remained normal throughout the repeat study. These curves (fig. 5) resemble those found in hematopoietic normals. In T.R., continued on busulfan, the slope of the ascending limb of the curve is less steep and the maximum lower than in H.G. or the normals. Pre- and post-treatment studies in A.M. and A.A. are shown in figure 6. In the pre-treatment studies in both patients the leukocyte counts were stable at a high level. Both were relapsing during the post-treatment studies and the peripheral white cell counts were rising rapidly. In each patient the rate of increase in label concentration is the same in the pre- and post-treatment studies.

The mean of nine differential cell counts done during each of these two studies in A.A. showed before treatment: 11.5 per cent myelocytes, 26 per cent metamyelocytes, 59 per cent segmented granulocytes, and 3 per cent lymphocytes; and after treatment: 3.5 per cent myelocytes, 11.5 per cent metamyelocytes, 73 per cent segmented granulocytes, and 11.5 per cent lymphocytes. Blasts and promyelocytes constituted less than 1 per cent in both studies. No consistent relationship between the curve of leukocyte DNA-P specific activity and the minor daily variations in differential cell counts was noted.

One patient with myelofibrosis, documented by histologic examination of marrow obtained by open biopsy, was studied at a time when he showed marked leukocytosis with many immature, but alkaline phosphatase-positive
Table 1.—Summary of Hematologic Data from Patients Studied

<table>
<thead>
<tr>
<th>Patient</th>
<th>Hb Gm.</th>
<th>VP RC %</th>
<th>Platelets $10^9$/mm.$^3$</th>
<th>WBC $10^9$/mm.$^3$</th>
<th>Differential</th>
<th>N-RBC/100 WBC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>B</td>
<td>P</td>
<td>M</td>
</tr>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>G. A. B.</td>
<td>13</td>
<td>40</td>
<td>—</td>
<td>6.7</td>
<td>71</td>
<td>1</td>
</tr>
<tr>
<td>C. T.</td>
<td>12</td>
<td>36</td>
<td>273</td>
<td>6.1</td>
<td>64</td>
<td>2</td>
</tr>
<tr>
<td><strong>Polycythemia vera</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K. T.</td>
<td>17.6</td>
<td>57</td>
<td>660</td>
<td>15</td>
<td>69</td>
<td>5</td>
</tr>
<tr>
<td>A. C.</td>
<td>19</td>
<td>62</td>
<td>500</td>
<td>16</td>
<td>88</td>
<td>1</td>
</tr>
<tr>
<td><strong>Chronic lymphocytic leukemia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. W.</td>
<td>6.8</td>
<td>24</td>
<td>125</td>
<td>167</td>
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<td></td>
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<tr>
<td>G. B.</td>
<td>11</td>
<td>31</td>
<td>52</td>
<td>68.5</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>S. W.</td>
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<td>16</td>
<td>82</td>
<td>291</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>L. R.</td>
<td>9.9</td>
<td>31</td>
<td>57</td>
<td>17.5</td>
<td>1</td>
<td></td>
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<tr>
<td>G. F.</td>
<td>15</td>
<td>46</td>
<td>337</td>
<td>108</td>
<td>1</td>
<td>14</td>
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<tr>
<td>T. R.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>before Rx</td>
<td>11</td>
<td>34</td>
<td>135</td>
<td>238</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>after Rx</td>
<td>13.7</td>
<td>43</td>
<td>—</td>
<td>9</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>H. G.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>before Rx</td>
<td>7.2</td>
<td>25</td>
<td>120</td>
<td>427</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>after Rx</td>
<td>10.7</td>
<td>37</td>
<td>225</td>
<td>6.4</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>A. M.</td>
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<td></td>
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<tr>
<td>before Rx</td>
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<td>20</td>
<td>1000</td>
<td>222</td>
<td>3</td>
<td>21</td>
</tr>
<tr>
<td>after Rx</td>
<td>8.4</td>
<td>28</td>
<td>990</td>
<td>28</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>A. A.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>before Rx</td>
<td>13.4</td>
<td>44</td>
<td>277</td>
<td>107</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>after Rx</td>
<td>13</td>
<td>40</td>
<td>232</td>
<td>24</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td><strong>Myeloblasts</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H. P.</td>
<td>6</td>
<td>16</td>
<td>90</td>
<td>52</td>
<td>1</td>
<td>6</td>
</tr>
</tbody>
</table>

Hb = hemoglobin; VP RC = volume of packed red cells; WBC = white blood cell count; B = blast; P = promyelocyte; M = myelocyte; Mm = metamyelocyte; Seg = segmented neutrophil; Eos = eosinophil; Baso = basophil; L = lymphocyte; Mo = monocyte; N-RBC = nucleated red blood cell.
Fig. 1.—Clearance of $^{32}$P from plasma of A. C. (untreated polycythemia vera) after intravenous injection of 2.0 μc. $^{32}$P/Kg. granulocytes. The curve of circulating leukocyte DNA-P specific activity obtained is indistinguishable from those found in patients with chronic granulocytic leukemia (fig. 7).

DISCUSSION

In the normal steady state, granulocytes are formed exclusively in marrow by cell division and maturation. Two physiologic compartments exist in marrow; the mitotic compartment, containing dividing cells and those that may divide, and the post-mitotic compartment, composed of non-dividing cells that will presumably mature and exit or die in situ. The introduction of label into DNA occurs only in cells of the mitotic compartment during DNA synthesis preparatory to mitosis. The persistence for days of a significant concentration of label in the body phosphate pools after intravenous injection of only 2 μc. $^{32}$P/Kg. appears to exclude the concept of "flash labeling" as a realistic basis for the mathematical analysis of DNA-P specific activity curves. Although continued labeling introduces mathematic complications, it may prove ultimately to be an asset in minimizing the effect, if any, of label reutilization. Recently, Feinendegen et al. have demonstrated that label reutilization may be a significant problem in studies using tritiated thymidine.28

Cells obviously must exit from marrow to enter the intravascular pool. Studies with diisopropyl fluorophosphate ($^{32}$P) labeling29 indicate there are
Fig. 2.—Specific activity of circulating leukocyte DNA-P in two hematologic normals (G. A. B. and C. T.) after 2.0 μc. P₃₂/Kg.; and specific activity of circulating leukocyte DNA-P after 2.0 μc. P₃₂ Kg. in three patients with chronic lymphocytic leukemia compared with mean curve from normals.

Fig. 3.—Specific activity of circulating leukocyte DNA-P after 2.0 μc. P₃₂ Kg. in two patients with polycythemia vera (K. T. and A. C.).
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Table 2.—Counts per Minute per μg. of DNA-P of Circulating Leukocytes from a Patient with Chronic Lymphocytic Leukemia after a Tracer and Therapeutic Dose of P³²

<table>
<thead>
<tr>
<th>Day after P³² Injection</th>
<th>Counts per Minute/μg. DNA-P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.0 μc./Kg.</td>
</tr>
<tr>
<td>1</td>
<td>0.3</td>
</tr>
<tr>
<td>3</td>
<td>0.6</td>
</tr>
<tr>
<td>5</td>
<td>0.6</td>
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<tr>
<td>7</td>
<td>1.2</td>
</tr>
<tr>
<td>9</td>
<td>1.6</td>
</tr>
<tr>
<td>11</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Table 3.—Counts per Minute per μg. of P of Acid-soluble (AS), Phospholipid (PL), Ribonucleic Acid (RNA), and Deoxyribonucleic Acid (DNA) Fractions of Circulating Leukocytes from a Patient with Chronic Lymphocytic Leukemia given 2.0 μc. P³²/Kg.

<table>
<thead>
<tr>
<th>Minutes after P³² Injection</th>
<th>Cell Fractions (CPM/μg. Phosphorous)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AS</td>
</tr>
<tr>
<td>35</td>
<td>0.5</td>
</tr>
<tr>
<td>100</td>
<td>0.4</td>
</tr>
<tr>
<td>195</td>
<td>0.3</td>
</tr>
</tbody>
</table>

about twice the number of intravascular granulocytes as can be calculated from leukocyte counts and blood volume. Those cells not actively circulating are presumed to be margined along vessel walls and are in very rapid equilibrium with those circulating. Normal granulocyte circulating half-time in man appears to be about 6–7 hours with exit from the circulation a random process. Available evidence indicates that once granulocytes exit from blood, they do not reenter in significant numbers. Thus, samples of leukocytes obtained from blood at daily intervals would each time represent an essentially new population because of replacement with new elements over 4 half-times.

The low level of label in circulating leukocyte DNA-P during the first 4–5 days in normals is compatible with the degree of labeling of lymphocytes. The 6-day delay in normals before the sharply rising concentration of label in circulating leukocyte DNA-P appears (fig. 2) probably reflects the time required for all the cells in the post-mitotic compartment at the time of label injection to pass through this compartment. Evidence for such a post-mitotic reservoir of considerable magnitude is ample. Thus, in normal man, this compartment is large enough to provide replacement for cells lost from the circulation for 5–6 days. Progress through it must be orderly, and release into blood largely a function of the time of entry of the cells from the mitotic compartment. Onset of the sharply ascending portion of the curve of circulating leukocyte DNA-P specific activity marks the beginning influx of cells formed after injection of label. The subsequent regression from the maximum level of specific activity must reflect continued replacement by less heavily labeled cells formed from a precursor pool containing a continuously decreasing concentration of label.
Fig. 4.—Specific activity of circulating leukocyte DNA-P after 2.0 μc. P³²/Kg. in five patients with chronic granulocytic leukemia, untreated or in relapse and on no therapy.

Fig. 5.—Specific activity of circulating leukocyte DNA-P after 2.0 μc. P³²/Kg. in two patients with chronic granulocytic leukemia in remission compared with mean curve from normals.

The size of the post-mitotic granulocyte compartment may be calculated from the number of cells leaving the circulation daily as determined by the DFP²⁻ technic (assuming significant re-entry does not occur) and the time required for orderly transit through the post-mitotic reservoir before entry into the blood. Athens et al.³⁸ found that in a 70 Kg. man, $1.26 \pm .52 \times 10^{11}$ granulocytes leave the circulation daily. A post-mitotic compartment with
Fig. 6.—Specific activity of circulating leukocyte DNA-P after 2.0 μc. P³²/Kg. in two patients with relapsing chronic granulocytic leukemia. In each patient the leukocyte counts shown were determined during the course of the post-treatment study when relapse was occurring. During the time of the pretreatment studies, leukocyte counts were stable at a much higher level.

A 6-day supply would contain $4.44 \times 10^{11}$ to $1.1 \times 10^{12}$—figures in good agreement with Osgood's calculations. Further, one or less divisions per day by the $3 \times 10^{11}$ cells computed to compose the mitotic pool would replace the daily loss of cells, a not unreasonable rate of division in light of the generative cycle time of marrow cells. Should $3 \times 10^{11}$ cells divide each day there would be a surplus nearly equal to the daily requirement. Prompt death and dissolution would be the only fate allowable for such an excess of cells in this system under steady state conditions. Pattee has recently presented evidence that in dogs, up to 60 per cent of newly formed myelocytes die without undergoing maturation.

The duration of granulocyte sojourn in tissues and cavities after exit from blood has been a matter of speculation because no direct way of examining the problem has been devised. Osgood deduced the presence of a large tissue pool of granulocytes to satisfy mathematical relationships evolving chiefly from the length of life of mature granulocytes in vitro. But adverse circumstances in vivo could well shorten their survival and the number of granulocytes postulated to be in tissues would be reduced proportionately. In previous studies in rabbits, the post-mitotic granulocyte reservoir was calculated to contain 20 times the number of intravascular granulocytes. This represented the total body store of extravascular post-mitotic granulocytes available to enter or re-enter the circulation, since production had been...
Myelofibrosis

Fig. 7.—Specific activity of circulating leukocyte DNA-P after 2.0 μc. P¹³²/Kg. in one patient with myelofibrosis compared with curve from T. R.

...supplied by nitrogen mustard. This mass of post-mitotic granulocytes approximates that calculated to be in the marrow. These findings do not exclude the existence of a large tissue pool, but do intimate that movement from it back into blood is insignificant. If, then, significant recirculation does not occur, the size of the tissue pool and the length of stay in tissues will not affect the preceding considerations of granulocyte kinetics. Whatever the size of the tissue pool, in the steady state the numbers of granulocytes leaving it are, on the average, equivalent to the number leaving the circulation.

Polycythemia Vera

The circulating leukocyte DNA-P specific activity curves obtained in patients with polycythemia vera did not differ appreciably in configuration from those of normals. This implies that, in this disorder, production, maturation, and transit of granulocytes are qualitatively similar to the normal. Demonstration of an increased intravascular pool in patients with polycythemia vera¹⁸,³⁰ excludes redistribution between circulating and margi- nated pools as the cause of the persistent granulocytosis. Athens and colleagues³⁰ have shown that despite a slight increase in the circulating half-time, the net blood granulocyte turnover was, on the average, approximately 5 times normal. Excluding significant recirculation of granulocytes, this requires about 5 times the normal supply from the post-mitotic compartment. The data reported here indicate that transit time through this compartment in patients with polycythemia vera is about the same as in normals, perhaps slightly shorter. Since, therefore, the fraction of the post-mitotic compart- ment moving into the blood per unit time is approximately equal to that of the normal, the size of the compartment in these patients has to be proportionately increased to provide for the increased supply of cells. Such an increase must result from the production of more cells; this could be brought
about by an increase in the number of cells that divide at a normal rate, by
a normal number of dividing cells dividing more rapidly, or by decreased
death of cells normally formed and their subsequent maturation. The data
do not permit selection of any one of these three possibilities.

**Chronic Lymphocytic Leukemia**

The very low concentration of label achieved in the circulating leukocyte
DNA-P in patients with chronic lymphocytic leukemia contrasts sharply with
the labeling curves obtained in all other groups. It has been suggested already
that the higher concentration of label found in patient L.R. was caused by
the presence of granulocytes. Christensen and Ottesen\(^4\) reached a similar
conclusion and deduced further that in their patients with chronic lympho-
cytic leukemia, the circulating lymphocyte DNA renewal rate approximated 0.1
per cent per day. Table 3 shows the very low rate of influx of label into various
fractions of the circulating lymphocytes of patient S.W. during a time when
the level of activity in plasma phosphate was falling from about 1100 to 50
cpm/µg, and contrasts strikingly with the rapid entry of phosphate into
marrow cells.\(^19\) It is unlikely, therefore, that very much DNA is formed
in lymphocytes circulating in the blood of patients with chronic lymphocytic
leukemia.

Alterations in kinetics, if any, produced by 20 µc. of P\(^{32}\)/Kg. were not
appreciated in this study for at least 10 days. Significant changes in the total
and differential leukocyte count did not occur, and circulating leukocyte
DNA-P specific activity (table 2) approximated 10 times that obtained with
2 µc. P\(^{32}\)/Kg.

These studies have not shown striking fluctuations in the concentration of
label in circulating leukocyte DNA of patients with chronic lymphocytic
leukemia, as did those of Perry et al.\(^17\) The explanation for this difference is
not apparent.

**Chronic Granulocytic Leukemia**

The curves of circulating leukocyte DNA-P specific activity reported here
confirm the departure from the normal reported previously.\(^6,7\) Circulating
leukocyte DNA-P specific activity increases progressively from the time of
label injection such that the low, flat segment found in normals, if represented
at all, is obscured. There is a suggestion of an increase in the rate of label
accretion between days 5 and 6 that, if real, could represent an entry into
the circulation of cells formed soon after label injection and released at about
the normal time. Thus, a population of granulocytes with essentially normal
kinetic behavior may be present in these patients.

Although the presence in the circulation of immature cells (cells engaged in
DNA synthesis and presumably replication) has been well documented,\(^8,41\)
it is not yet clear that these are the sole or even the principal contributors to
the progressive rise in circulating leukocyte DNA-P specific activity during
the first 3 to 4 days after label injection. Bond et al.\(^41\) demonstrated with
radioautography in cultures of blood from patients with chronic granulocytic
leukemia that DNA synthesis occurred in 50 per cent of cells up through
the myelocyte stage; Osgood et al., using P, found the rate of DNA syn-
thesis in circulating cells in vitro only one-fifth the apparent rate in vivo.
If this lower rate represented the capacity of circulating cells to synthesize
DNA in vivo, the progressive rise in circulating leukocyte DNA-P specific
activity in these patients would result chiefly from DNA formed in cells
outside the circulation. It is necessary to recognize, as pointed out by Osgood,
that several factors would tend to minimize both the apparent and actual
rate of DNA synthesis in cultures of the circulating cells. Thus, at present, all
that can be deduced with certainty is the circulation of a significant number
of cells that have synthesized DNA within 1 day of label injection.

Lala et al., from data similar to that reported in this study, computed the
“average survival” for leukemic granulocytes to be $4.2 \pm 0.3$ days, agreeing
well with the determination of Osgood and Krippaehne. Their calculations
were based on the assumption that changes in circulating leukocyte DNA
activity were due to a random entry of labeled cells formed from a decaying
precursor pool. The second part of the assumption is no doubt valid, but
the evidence for the random entry of labeled cells is not conclusive. Although
the concept that in chronic granulocytic leukemia the blood and extravas-
cular leukopoietic compartment(s) represent a single compartment may be
attractive, the evidence for this is scant. In fact, a recent study by Clarkson
et al. refutes this by demonstrating differences in the per cent of labeled
cells in blood and marrow after infusion of tritiated thymidine into the splenic
artery. Killman et al also deduce some regulation of release of “blasts” from
marrow into blood in patients with chronic granulocytic leukemia in a termi-
nal, acute phase of the disease.

If the leukemic mature granulocyte is short-lived, the relatively normal
generative cycle time and pace of maturation require that the pool of pro-
iferating cells be increased to provide the increased numbers of cells found
in this disease. Comparison of the relative proportions of myeloid elements
found in normal marrow with the proportions we have found in the circula-
tion of 39 untreated persons with chronic granulocytic leukemia reveals,
respectively: blasts 1.8 and 4.6 per cent, promyelocytes 5.9 and 5.4 per cent,
myelocytes 28.7 and 22.3 per cent, and metamelocytes and mature polymor-
phonuclears 63.5 and 67.7 per cent. Thus, only the blasts are disproporti-
ately increased, assuming blood and extravascular leukopoietic compartments
in chronic granulocytic leukemia have similar differential counts.

The results of studies in two patients in remission after treatment with
busulfan (fig. 6) show the return toward normal reported previously by Perry. The curve for H.G. would likely not be distinguished from the normal
without prior knowledge of her disease. In patient T.R., however, even
though release of labeled cells from the post-mitotic compartment appears
to begin at about the same time, the ascending slope is considerably less steep
and the maximum reached is lower and occurs later than in H.G. These
differences in the labeling curve in T.R. during remission could be effected
by alteration in the characteristics of labeling in the proliferating pool caused
by busulfan. A decrease in the concentration of label achieved in the proliferating cells would lower the maximum found in circulating cells. Changes from the normal in degree of labeling between succeeding generations, however, would be needed to alter the time of the maximum, if progression through the post-mitotic compartment and the intravascular time were normal. Prolongation of the circulating half-time could decrease the slope of the ascending curve and delay the time of the maximum, but should not decrease its level if, in the proliferating pool, the concentration of label in DNA reaches the value attained in normals.

Figure 6 shows the results of studies done in two patients in only partial remission but on no therapy. In spite of significant increases in the concentration of circulating leukocytes during the study, the slopes of the curves in both instances are not significantly different from curves obtained when they were in full relapse with a high but essentially unchanging concentration of circulating leukocytes. This emphasizes the importance of the characteristic of label incorporation from a decaying precursor into a proliferating pool, and indicates that this factor must be considered along with cell movements in order to understand the meaning of leukocyte DNA specific activity curves.

At a time when the clinical picture was indistinguishable from that of chronic granulocytic leukemia, the leukocyte DNA-P specific activity curve obtained in a patient with myelofibrosis resembles in all respects those found in persons with chronic granulocytic leukemia, suggesting similar disturbances in leukocyte kinetics.

**Summary**

Leukocyte physiology in the normal and in hematopoietic disease states in humans has been studied by DNA-P labeling with inorganic P\(^{32}\). In the normal there is a post-mitotic granulocyte reservoir in marrow about 17 times the size of the intravascular compartment. Progress through this reservoir is orderly and requires about 6 days. Some 1-2 \(\times 10^{11}\) cells daily are released from it into the blood. In polycythemia vera there is an increased production of granulocytes. DNA-P labeling in patients with chronic lymphocytic leukemia occurs at a very low level and is compatible with a very slow rate of cell renewal. In one patient with chronic lymphocytic leukemia, no disturbances in kinetics caused by a dose of 20 \(\mu\)c. of P\(^{32}\)/Kg. were detected during the time of the study. Although a progressively rising concentration of label in circulating leukocyte DNA was found in patients with chronic granulocytic leukemia, without the lag suggesting a distinct marrow phase, it is concluded that the blood and extravascular leukopoietic compartments cannot be a single compartment. An essentially normal curve is obtained after induction of a complete remission with busulfan.

**Summario in Interlingua**

Le physiologia del leucocytos human in statu normal e in le statos de morbo hematopoietic esseva studiate per medio del marcation de acido deoxyribonucleic con inorganic phosphoro radioactive. In subjectos normal
il existe in le medulla un reservoir de post-mitotic granulocytos de circa 17 vices le magnitude del total del granulocytos intravascular. Le transition a transverso iste reservoir es es systematic e require circa 6 dies. Circa 1 a $2 \times 10^{11}$ cellulas es liberate per die ab le reservoir ad in le sanguine. In polycythemia ver il ocorre un augmento del production de granulocytos. In patientes con chronic leucemia lymphocytic le marcation del acido deoxyribonucleic con phosphoro occurree in hassissime mesura e corresponde a un lentissime renovage cellular. In un patiente qui habeva chronic leucemia lymphocytic, nulle disturbationes del cinetica esseva causate per un dose de 20 $\mu$C de $^{32}$P per kg de peso corporee secundo le effortio de deteger tales durante le tempore del studio. Ben que on progressive augmento esseva trovate in le concentration de radioactive marcation in le leucocytos in le circulation in patientes con chronic leucemia granulocytic, sin signos de un retardo suggestionante un distincte phase medullari, le conclusion es formulate pie le sanguine e le compartimentos de leucopoiese extravascular non pote esser un sol compartimento. Un essentialmente normal curva es obtenite post le induction de un complete remission con busulfan.

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LEUKOCYTE KINETICS STUDIED BY DNA-PHOSPHORUS

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Leukocyte Kinetics in Hematologic Disorders Studied by DNA-Phosphorus Labeling

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