CHARACTERISTICS OF CELL PROLIFERATION IN FOUR PATIENTS WITH UNTREATED ACUTE LEUKEMIA

By ALVIN M. MAUER AND VIRGINIA FISHER

EARLIER CONCEPTS of a rapidly growing malignant cell population in patients with acute leukemia have been challenged by the finding of relatively few mitotic figures among leukemic cells. A cell label, tritiated thymidine (H3T) has since become available with which further studies of the characteristics of cell proliferation in patients with acute leukemia can be done. The following report is of studies with this label in four children with untreated acute leukemia. A preliminary communication concerning one of these patients has already been published, and studies in another two have presented briefly.

PATIENT MATERIAL AND METHODS

All four patients were from The Children's Hospital. None had received any treatment whatsoever at the time of study. Information concerning the patients and their laboratory values are given in Table 1. Patient 1 had Downs syndrome confirmed by chromosomal analysis. Patients 1 and 3 had myeloblastic leukemia and Patients 2 and 4 had lymphoblastic leukemia.

The labeling injection of H3T was given intravenously in a dose of 200 μg/Kg body weight. The H3T was obtained from New England Nuclear Corporation and had a specific activity of 360 mc. per mM. Patients 1 and 2 were given a single injection of label at 3:30 p.m. and 11:45 a.m., respectively. Beginning 1 hour after injection, serial blood and bone marrow samples were obtained. Patients 3 and 4 were given injections of label at 10-hour intervals beginning at 12:25 p.m. and 12:45 p.m., respectively. Blood and bone marrow samples were obtained 1 hour after the first injection and immediately before and 1 hour after subsequent injections.

Seven months after the initial study in Patient 1, a second study was done during a subsequent relapse. A single injection of H3T was given and bone marrow was obtained from six different sites beginning 60 minutes after the labeling procedure. She was receiving no therapy at the time of this study.

Autoradiographs were prepared from the blood and bone marrow samples with Kodak-AR10 stripping film. After a suitable exposure period the autoradiographs were developed and stained. From 4000 to 6000 nucleated cells were observed from each sample and the number of labeled cells and the grain count above each cell were determined.

RESULTS

In order to determine the distribution of label in the bone marrow of a...
leukemic patient, six marrow sites were aspirated beginning 1 hour after the injection of H3T into Patient 1. The marrow was aspirated from four dorsal and lumbar vertebral spines and the left and right anterior iliac crests. All marrow samples were obtained within 15 minutes. Ten-thousand cells were counted from the autoradiographs prepared from each sample.

The results of the study are shown in Table 2. The per cent of labeled leukemic cells and their mean grain count were quite similar. This label was thus evenly distributed throughout the marrow sites sampled. In the following studies the marrow sites sampled were the dorsal and lumbar vertebral spines and the anterior and posterior iliac crests.

**Single Injections of Label**

In Patients 1 and 2 a single injection of label was given followed by serial blood and bone marrow samples. In Figure 1 is shown the time course for labeled cells in the bone marrow of Patient 1. One hour after injection 5.5 per cent of the leukemic cells were labeled. The mean grain count of labeled cells was 30. Thereafter, an increase in the per cent of labeled cells occurred, associated with a decreasing mean grain count. By 8 hours after the injection, the per cent of labeled cells had doubled. Beginning 16½ hours after injection, a second increase in the per cent of labeled cells occurred, associated with a further decrease in mean grain count. By 28½ hours after the injection the per cent of labeled cells had redoubled. Thereafter, less frequent samples were obtained but some further increase in the per cent of labeled cells was observed, although the mean grain count remained the same. A transfusion of 80 ml packed red blood cells was given after the eighth marrow sample had been obtained.

In order to determine the time course for appearance of labeled mitotic figures, 100 mitotic figures from each marrow sample were counted and the
Table 1.—*Patient Information and Laboratory Values*

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yrs.)</th>
<th>Sex</th>
<th>Wt. (Kg.)</th>
<th>Day of Study</th>
<th>Hemoglobin (Gm. %)</th>
<th>WBC (No./mm.³)</th>
<th>Leukemic Cells (%)</th>
<th>Platelets (No. x 10⁹/mm.³)</th>
<th>Therapy</th>
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<tr>
<td>1</td>
<td>2½</td>
<td>F</td>
<td>10</td>
<td>1</td>
<td>3.4</td>
<td>48,350</td>
<td>90</td>
<td>20</td>
<td>80 ml. blood at 9:00 p.m.</td>
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<td></td>
<td></td>
<td>2</td>
<td>3.1</td>
<td>30,600</td>
<td>92</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td></td>
<td></td>
<td>3</td>
<td>4.4</td>
<td>13,800</td>
<td>85</td>
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<td>150 ml. packed RBC at 1:00 p.m.</td>
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<td>4</td>
<td></td>
<td></td>
<td>4</td>
<td>8.5</td>
<td>2,700</td>
<td>34</td>
<td>—</td>
<td>50 ml. packed RBC 2:00 p.m.</td>
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<td></td>
<td>(Study II)</td>
<td></td>
<td></td>
<td></td>
<td>10.1</td>
<td>9,450</td>
<td>30</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>F</td>
<td>16.4</td>
<td>1</td>
<td>7.3</td>
<td>7,650</td>
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<td>86</td>
<td>500 ml. blood at 3:45 p.m.</td>
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<td>4</td>
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</tr>
<tr>
<td>3</td>
<td>8½</td>
<td>M</td>
<td>27</td>
<td>1</td>
<td>12.6</td>
<td>5,200</td>
<td>24</td>
<td>108</td>
<td>6-mercaptopurine, 75 mg. daily</td>
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<tr>
<td>4</td>
<td>11</td>
<td>M</td>
<td>59</td>
<td>1</td>
<td>10.1</td>
<td>1,750</td>
<td>4</td>
<td>56</td>
<td>Prednisone, 60 mg. and 6-mercaptopurine, 75 mg. daily</td>
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<td></td>
<td></td>
<td>5</td>
<td>10.1</td>
<td>1,850</td>
<td>0</td>
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presence or absence of overlying grains noted. The results of the study are shown in Figure 2. One hour after the injection no mitotic figures were labeled. By 6 hours 94 per cent of the mitotic figures were found to be labeled. Thereafter there was a decrease in the per cent of labeled mitotic figures, and at 16½ hours 60 per cent of the mitotic figures were labeled. Once again an increase in the per cent of labeling was found, and at 24½ hours 98 per cent of the mitotic figures were labeled. In subsequent samples the per cent of labeled mitotic figures once again decreased.

The leukemic cells in these marrow samples varied in size. It was noticed that in the first sample only the larger leukemic cells having a fine nuclear chromatin pattern were labeled. Small leukemic cells whose chromatin pattern was somewhat more coarse were unlabeled. In later samples labeled small leukemic cells appeared. Therefore, the leukemic cells were divided into large and small cell compartments and the per cent of labeled cells and their mean grain count determined for each group. Labeled large cells found in the 1-hour sample are shown at the top of Figure 3, and labeled small cells which were present in the 24½ hour sample are shown at the bottom of Figure 3.

<table>
<thead>
<tr>
<th>Minutes after Injection</th>
<th>Site of Aspiration</th>
<th>Per Cent Labeled Blasts</th>
<th>Mean Grain Count</th>
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<tr>
<td>60</td>
<td>Vertebral spine</td>
<td>15.6</td>
<td>13</td>
</tr>
<tr>
<td>61</td>
<td>Vertebral spine</td>
<td>12.4</td>
<td>11</td>
</tr>
<tr>
<td>62</td>
<td>Vertebral spine</td>
<td>15.7</td>
<td>12</td>
</tr>
<tr>
<td>63</td>
<td>Vertebral spine</td>
<td>15.9</td>
<td>14</td>
</tr>
<tr>
<td>65</td>
<td>Left anterior iliac crest</td>
<td>15.0</td>
<td>15</td>
</tr>
<tr>
<td>75</td>
<td>Right anterior iliac crest</td>
<td>15.5</td>
<td>14</td>
</tr>
</tbody>
</table>

Fig. 2.—Per cent of labeled mitotic figures in the bone marrow of Patient 1 after single injection of \(H_3\) thymidine.
Fig. 3.—Autoradiographs of bone marrows from Patient 1. In the upper two frames only large leukemic cells were labeled 1 hour after the injection of label. In the lower two frames small leukemic cells are also labeled in the marrow sample obtained 24½ hours after the injection of label.

An average of 12 per cent of the leukemic cells were large, with a range from 10 to 14 per cent in the various samples. During the study period no change in the ratio of large to small cells occurred. The percentages of labeled large and small leukemic cells are shown in Figure 4. Initially about one-half of the large cells were labeled. Transient increases in the per cent of labeled large cells were found maximal at 6 hours and again at 20½ hours. By 41½ hours, however, a definite decrease in the per cent of labeled large cells had occurred. Initially, no labeled small cells were found. Thereafter, an increase in the per cent of labeled small cells was observed, particularly during the second day after the injection of label.

The change in mean grain count of the labeled cells in these two compartments is shown in Figure 5. Although a progressive decrease in mean grain count was observed in the labeled large cells, no significant change was found in the mean grain count of labeled small cells.

In Figure 6 is shown the time course for labeled cells in the bone marrow of patient 2. One hour after the injection 6.6 per cent of the leukemic cells were labeled. The mean grain count of the labeled cells was 13.3. Thereafter, an increase in the per cent of labeled cells occurred, associated with a decreasing mean grain count. Six hours after the injection, the per cent of labeled cells was 11.5, almost double the per cent initially found. At the time of the sample obtained 22 hours after the injection, a further increase in the per cent of
labeled cells was occurring, associated with a decrease in mean grain count of labeled cells. Thirty hours after the injection the per cent of labeled cells was 22.5, almost redoubling the per cent of labeled cells. A transfusion of 500 ml. whole blood was given after the eighth marrow sample had been obtained.

One hundred mitotic figures were counted from each marrow sample and the per cent of labeled figures was determined. The results of the study are shown in Figure 7. One hour after the injection no labeled mitotic figures were found. By 6 hours 85 per cent of the mitotic figures were labeled. At 10½ hours 64 per cent of the figures were labeled, and at 22 hours 78 per cent of the figures were found to be labeled. Thereafter, the per cent of labeled mitotic figures decreased.

In this patient the leukemic cells were also divided into large and small cell compartments and the per cent of labeled cells and their mean grain count determined for each group. An average of 12 per cent of the leukemic cells were large, with a range from 9.4 to 13 per cent in the various samples. During the period of study no change in the ratio of large to small cells occurred.

The per cent of labeled large and small leukemic cells is shown in Figure 8. Initially about one-half of the large cells were found to be labeled. Transient increases in the per cent of labeled large cells occurred, maximal at 6 hours and again at 26 hours. During the course of the study, however, a progressive
A decrease in the per cent of labeled large cells was observed. Initially no labeled small cells were found. Some labeled small cells were present in the 3-hour sample and an increase in the per cent of labeled small cells occurred thereafter.

The change in mean grain count of the labeled cells in these two compartments is shown in Figure 9. There was a progressive decrease in the mean grain count of the labeled large cells. The mean grain count in the small cells increased until 22 hours, when it thereafter decreased in a manner similar to the labeled large cells.

The appearance of labeled blast cells in the blood of these two patients is shown in Figure 10. One and 2 per cent of the blast cells, respectively, were found to be labeled initially. No change occurred in the concentration of labeled blast cells until 8 and 10 hours after the injection, when an increase in the per cent of labeled blast cells occurred at a rate of 10 to 15 per cent per day.

**Serial Injections of Label**

In the studies of patients 3 and 4 serial injections of $H_3T$ were given at 10-hour intervals. In order to determine the changes in cell labeling with these injections, marrow samples were obtained immediately before and 1 hour after the injections. The results of these studies are shown in Figure 11 and Table 3.

In Patient 3, shown on the left in Figure 11, 14.5 per cent of the cells were...
Fig. 6.—Per cent of labeled blast cells and their mean grain count in Patient 2 after single injection of H₃ thymidine.

Fig. 7.—Per cent of labeled mitotic figures in the bone marrow of Patient 2.
Fig. 8.—The change in the per cent of labeled large and small cells in Patient 2.

Fig. 9.—The mean grain count of labeled large and small cells in Patient 2.
labeled with the initial injection. An additional 7 per cent of the cells were labeled with the second injection. At the time of the third injection no additional cells were found to be labeled, but there was a striking increase in the mean grain count of the labeled cells.

Similar results were found in Patient 4, shown on the right in Figure 11. With the initial injection, 12.2 per cent of the leukemic cells were labeled. An additional 7.7 per cent of the cells were labeled with the second injection. Again at the time of the third injection no additional cells were found to be labeled, but an increase in the mean grain count of labeled cells occurred. With the fourth injection, an additional 5.1 per cent of the cells were found to be labeled.

In this patient it was possible to determine the characteristics of labeling in a normal cell population. About 5 per cent of his nucleated marrow cells were normoblasts. With each injection the per cent of labeled normoblasts increased until the time of the last injection, when almost all of the cells had already been labeled.

The labeling of leukemic cells in the blood and bone marrow of Patient 3 are compared in Figure 12. No such comparison was possible in Patient 4 because of the paucity of blood leukemic cells. Initially, only 1.5 per cent of the blood leukemic cells were labeled in Patient 3. Thereafter, an increase in the per cent of labeled cells was observed similar to that seen in Patients 1 and 2, as shown in Figure 10. The change in the per cent of labeled leukemic blast cells...
Fig. 11.—Per cent of labeled blast cells and their mean grain count in Patients 3 and 4 during injections of H₃T at 10-hour intervals.

of the blood did not show the relationship to injections of label that was seen in the bone marrow cells.

In these two patients the per cent of labeled mitotic figures was 86 and 98 per cent, respectively, by the end of the study period.

Effect of the Labeling Procedure

In none of these patients was there any evidence of toxicity from the label. All these patients achieved a remission with therapy and did not appear to have any late irradiation effects from the tritiated thymidine. The survival of these patients after the studies is as follows: Patient 1, 734 months; Patient 2, 36 months; Patient 3, 29 months (still living); and Patient 4, 10½ months. All the patients had normal blood count values during their remissions.

DISCUSSION

The use of tritiated thymidine (H₃T) as a cell label in conjunction with autoradiographic technics in assessing characteristics of cell proliferation has been recently reviewed. This label is incorporated into the nuclei of cells in DNA synthesis as DNA-thymidilic acid. There is subsequently no label elution. After intravenous injection, an effective concentration of the label is available only for a period of 20 to 30 minutes for incorporation into the nuclei of those cells in the DNA synthesis phase of the mitotic cycle. A cohort of
CELL PROLIFERATION IN UNTREATED LEUKEMIA

Table 3.—Labeling of Leukemic Marrow Cells by Serial Injections of H3 Thymidine

<table>
<thead>
<tr>
<th>Sample</th>
<th>Patient 3</th>
<th>Patient 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Labeled Cells (%)</td>
<td>Mean Grain Count</td>
</tr>
<tr>
<td>1</td>
<td>14.5</td>
<td>26</td>
</tr>
<tr>
<td>2</td>
<td>11.6</td>
<td>19</td>
</tr>
<tr>
<td>3</td>
<td>18.6</td>
<td>24</td>
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<td>4</td>
<td>25.9</td>
<td>30</td>
</tr>
<tr>
<td>5</td>
<td>23.3</td>
<td>53</td>
</tr>
<tr>
<td>6</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>—</td>
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</tr>
</tbody>
</table>

dividing cells can thus be labeled. The label is divided between daughter cells during mitosis. Information concerning the characteristics of proliferation can be obtained by following this cohort of labeled cells by means of autoradiography through serial samplings of the cell population.

Two special problems arise, however, in the use of this label for the study of cell proliferation in acute leukemia. The marrow cell population had to be sampled serially in order to follow the time course of the labeled dividing cells. Therefore, it was necessary to determine if the label was evenly distributed throughout the marrow spaces to be sampled and if these marrow spaces would be representative of proliferative activity in all marrow cells. In Patient 1, even distribution of the label and consistent proliferative activity were found in the marrow spaces which were used in all of these studies.

The label H3T is not a natural precursor for DNA synthesis. An enzyme, thymidine kinase, is necessary for the preparation of the thymidine for incorporation as DNA-thymidilic acid. Some question has been raised about the presence of this enzyme in some tumor tissues and the availability of the label for all dividing malignant cells. Incomplete labeling of the leukemic cells in DNA synthesis might result if all cells did not possess sufficient thymidine kinase. In the data presented concerning the labeling of mitotic figures, almost all were found to contain tritiated thymidine—within 6 hours of the injection of the label in Patients 1 and 2, and by the end of the study period in Patients 3 and 4. Thus, in these patients almost all of the cells in DNA synthesis were demonstrated to be capable of incorporating the label. The small per cent of unlabeled mitotic figures found did not indicate a significant population of dividing cells which could not be labeled. From these observations, the label is an effective one for studying some aspects of cell proliferation in acute leukemia.

In the two patients given a single injection of H3T, only a small per cent, 5.5 and 6.6, of the marrow leukemic cells were found to be labeled after 1 hour. No mitotic figures were labeled at that time; therefore, the labeled cells represented those cells in DNA synthesis at the time of the injection. Subsequently, there was an increase in the per cent of labeled cells, associated with a decreasing mean grain count. By 6 to 8 hours the per cent of labeled cells had doubled and must have gone through their first mitotic division after...
Fig. 12.—Per cent of labeled blast cells in the bone marrow and blood of Patient 3 during injections of 131I at 10-hour intervals.
having incorporated the H$_2$T. Beginning at 16½ hours in Patient 1 and already in progress at the 22-hour sample in Patient 2, a further increase in the per cent of labeled cells was found, associated with a decrease in mean grain count. At 28½ and 30 hours, respectively, the per cent of labeled leukemic cells had essentially redoubled. Thus, a second mitotic division of the labeled cells must have occurred and the generation time of these dividing cells was therefore somewhere between 15 to 20 hours.

Confirmation of the two mitotic divisions was found in Patient 1 in the appearance of two waves of labeled mitotic figures which coincided with the two increases in per cent of labeled cells. The intervening appearance of unlabeled mitotic figures between the two peaks of labeled mitotic figures was not clearly observed in Patient 2. However, the time between the fourth and fifth samples was long in this patient and spanned the time when unlabeled mitotic figures were observed in Patient 1.

In both of these patients the per cent of labeled cells increased as a result of the mitotic divisions. The most likely explanation for this observation is that the dividing, labeled cells were increasing relative to a population of unlabeled, nondividing leukemic cells present in the marrow. There is no evidence to suggest that the labeled cells were increasing because of the loss of unlabeled cells to the blood. During this time labeled cells were also appearing in the blood. Thus, the marrow cell population of these patients apparently consisted of both proliferating and nonproliferating leukemic cells.

It was further possible to identify these cell populations morphologically, as Gavosto and his co-workers have also described. The only leukemic cells initially labeled were large with fine nuclear chromatin. The smaller leukemic cells with coarse nuclear chromatin did not incorporate the label, as apparently none of them were in the DNA synthesis phase of the mitotic cycle.

In both of these patients about one-half of the large cells were initially labeled and transient increases in per cent labeling were found during the two observed divisions of these cells. As time passed, the per cent of labeled large cells decreased, and they were replaced by unlabeled large cells. During this time the per cent of labeled small cells increased, and the only evident explanation for this observation is that the labeled large cells after one or more divisions became smaller and joined this nondividing population of cells.

Reutilization of label might be considered as a possible explanation for the appearance of labeled small cells. Significant labeling of this population by the availability of relatively small amounts of H$_2$T is unlikely if none of these cells could incorporate the label from the higher concentrations offered by intravenous injection.

Further evidence for the dividing nature of the large cells and nondividing nature of the small cells was found in the mean grain count in these two cell groups. The large cells exhibited the decreasing grain count characteristic of dividing cells as the label was shared between daughter cells. The mean grain count of the small cells did not decrease. The increasing per cent of labeled small cells must have reflected incoming labeled cells from the initially labeled
group of large cells as they underwent transformation to small, nondividing cells.

Further information concerning the generation time of dividing leukemic cells and the nonuniformity of proliferative capacity in the marrow cell population was obtained from studies in Patients 3 and 4. In these patients serial injections of label at 10-hour intervals were given. With the first and second injections, newly labeled cells appeared in the marrow cell population. However, in both patients at the time of the third injection, 20 hours after the first, no newly labeled cells were found, but increases in mean grain count of labeled cells were observed. Thus, at the 20-hour injection, the cells incorporating the label already contained label and must have been in DNA synthesis at the time of the first injection. The generation time for these cells, from one DNA synthesis period to the next, was about 20 hours. An unlabeled population of cells was in DNA synthesis in Patient 4 thirty hours after the first injection, and a further increase in per cent of labeled cells of 5.1 was found after the fourth injection.

In these two patients a considerable population of nondividing leukemic cells was also found. Although one full generation time for the dividing leukemic cells had been encompassed by the labeling period and almost all mitotic figures were found to be labeled, more than 70 per cent of the marrow leukemic cells remained unlabeled. These unlabeled cells must have been nonproliferative.

The time course of labeled leukemic cells in the blood is such as to suggest that the blood leukemic cells are essentially nonproliferative. The increasing per cent of labeled cells must have reflected selective release of mostly nondividing cells from bone marrow and possibly other tissue sites to blood. In Patients 1 and 2 the time courses for labeled leukemic cells of the blood and the marrow small cell compartment were similar. Measurements of cell proliferation in acute leukemia in which only blood cells are studied therefore would not completely assess the proliferative capacity of the leukemic cell population.

An increasing per cent of labeled cells at a time when the blood cell counts were fairly stable must indicate some turnover of the leukemic cell population of the blood. The fate of these cells cannot be deduced from these studies. The disappearing cells may be destroyed or may return to tissue sites, perhaps to become dividing cells once more. The turnover of leukemic cells through blood cannot be used as a measure of cell production rate at this time because of our ignorance concerning the complete life cycle of the leukemic cell.

Diurnal variation of marrow cell proliferation has been demonstrated in normal individuals. No diurnal variation in the per cent of blood leukemic cells incorporating H3T has been found. Blood leukemic cells are not the proliferating cell compartment, however, and the question of diurnal variation of proliferative activity must remain an open one. There is no evidence that any significant difference in the interpretation of these data would result.

The relatively small per cent of marrow leukemic cells incorporating H3T as well as the discrepancy between blood and bone marrow labeling have been
reported also in other studies.\textsuperscript{17-22} Support for the concept of a nonuniform leukemic cell population with respect to proliferative activity has been found by Killmann and co-workers in adult patients with acute leukemia.\textsuperscript{18,21,22} Their calculations of generation times for the dividing leukemic cells were 2 to 3 times longer than reported here. A generation time of 12 to 18 hours for dividing leukemic myeloblasts was assumed in studies of 3 adult patients by Monti and his co-workers.\textsuperscript{23} These data also indicated a nondividing group of cells. Clarkson and his co-workers\textsuperscript{24} found that 82 to 93 per cent of marrow leukemic cells became labeled in four patients in whom H\textsubscript{3}T was infused from 8 to 10 days. This interesting observation is perhaps not unexpected in view of the increase in per cent of labeled cells which occurred after a single injection in two of our patients.

The cell populations of some animal tumors also have been found to contain dividing and nondividing elements.\textsuperscript{25-27} Baserga\textsuperscript{27} found that the per cent of dividing cells decreases with the growth of the cell population in implanted mouse ascites tumors. If a similar relationship occurred in leukemia, then the ratio of dividing to nondividing cells might also vary with the growth of the cell population in these patients, and therefore change during the course of the disease.

From the data obtained in these studies a tentative model for the leukemic cell populations in these four patients can be constructed. A population of large leukemic cells was dividing with a generation time of 15 to 20 hours. These cells comprised about 12 per cent of the marrow leukemic cells in Patients 1 and 2 and a somewhat larger percentage in Patients 3 and 4 because of the initially greater per cent of labeled cells. After one or more mitotic divisions these cells became smaller and stopped dividing. Some of these cells then appeared in the blood of these patients. The bulk of marrow leukemic cells and almost all of the blood leukemic cells consisted of nondividing leukemic blasts.

As initially labeled cells joined this nondividing compartment, new unlabeled large cells appeared to take their place. The origin of these cells remains in question. The dividing cells could have arisen from continuing leukemic transformation of normal cells. However, the small, nondividing cells of blood and bone marrow could once again have enlarged to go through another cycle of cell division. In this latter case the leukemic cells could have been a closed, self-replicating population. No indication of a survival time for leukemic cells could be obtained from these studies.

The presence of a considerable population of nondividing leukemic cells has significance regarding therapy of this disease. Most chemotherapy for acute leukemia has been directed toward inhibition of a dividing cell population, and would be relatively ineffective against nondividing cells. Even if all cell division in the leukemic population were inhibited, a group of unaffected, nondividing cells might be left behind which could once again reinitiate proliferation actively as resistance to drug therapy was achieved. Some evidence for the persistence of leukemic cells in successfully treated patients in remission has been presented.\textsuperscript{28}
SUMMARY

The characteristics of proliferation of leukemic cells in four children with untreated acute leukemia have been studied. In all four of these children a population of marrow leukemic cells was found which were dividing with a generation time of about 15 to 20 hours. In two of these patients it was possible to demonstrate that these dividing cells after one or more mitotic divisions became smaller and stopped dividing. In all of these patients 70 percent or more of the leukemic cells of the marrow and almost all leukemic cells of the blood were nonproliferative at the time of these studies. These nondividing cells would be relatively unaffected by chemotherapeutic agents designed to inhibit cell division.

SUMMARIO IN INTERLINGUA

Esseva studiate le characteristica del proliferation de cellulas leucemic in quatro juveniles con nontractate leucemia acute. In omne he quatro patientes, un population de leucemic cellulas medullar esseva trovate ille quale se divideva con un tempore de generation de inter 15 e 20 horas. In duo del casos, il esseva possibile demonstrar que le cellulas in experientiar ille division deveniva plus micre post un o plure divisiones mitotic e postea cessava divider. In omne le patientes del serie, 70 pro cento o plur del cellulas leucemic del medulla e quasi omne le cellulas leucemic del sanguine esseva nonproliferative at tempore del hic-reportate studios. Tal cellulas non ingagiate in division active debe esser relativemente exempte del influentia de agentes chimotherapeutic destinate a inhibit le division cellular.

REFERENCES

Characteristics of Cell Proliferation in Four Patients with Untreated Acute Leukemia

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