Chronic Granulocytic Leukemia and the Philadelphia Chromosome

By P. H. Fitzgerald, Angela Adams and Frederick W. Gunz

In 1960 it was reported by Nowell and Hungerford12,14 and soon confirmed by Baikie et al.2 that an abnormal chromosome could be found in the leukocytes of some patients with chronic granulocytic leukemia. The abnormality occurred in one of the four smallest autosomes, an acrocentric, probably No. 21 in the Denver4 classification, and consisted in the loss, either by deletion or translocation, of approximately half its long arm, thus leaving a structure noticeably smaller than the three other small acrocentrics. In line with the recommendations of the Denver conference, the abnormal chromosome was termed Ph1, after Philadelphia, the city in which it was first seen. The Philadelphia13,15 and Edinburgh2,18 workers have among them described details of 30 cases of chronic granulocytic leukemia, in 24 of which the Ph1 was seen. To these we now add another 12, four of which have already been briefly reported.1,5,6 In studying them, we aimed at obtaining information on some hitherto obscure points concerning the chromosome abnormality, particularly the following:

1) How constantly does Ph1 occur in chronic granulocytic leukemia?
2) What are the best means of demonstrating the abnormality?
3) What is the effect of treatment of the leukemia on the frequency of Ph1?
4) What is the significance of the abnormality?

These questions will be discussed following the presentation of our findings.

Materials and Methods

Studies were carried out on the blood and marrow of 12 consecutive patients with chronic granulocytic leukemia, six men and six women aged 25 to 67 years. Cases 1, 2, 9 and 11 were newly diagnosed and untreated when first studied; the others had received either radio- or chemotherapy or both (table 1). Two patients, Nos. 4 and 5, were in an acute "blastic" exacerbation. Repeated examinations were made at different stages of the disease where possible. Our findings are based on a study of 19 preparations of the blood from 11 patients containing 698 metaphase figures, and of 15 marrow preparations from 10 patients containing 526 metaphase figures, or altogether on 1224 mitoses in which the chromosomes could be accurately counted.

Blood was obtained by venepuncture and heparinized. Leukocytes from all blood specimens except that of Case 2 were cultured and prepared for chromosome study by the method of Moorhead et al.,12 modified in minor details. The culture time varied from 2 to 4 days as indicated in table 1. Leukocytes from Case 2 were examined by a direct method (Fitzgerald5). Air-dried films from all cases were stained either by Giemsa or acetic acid-orcein.

Marrow was obtained by sternal or posterior iliac puncture. All the marrow specimens were examined by direct methods, without culture. Part of specimen 1/8/62 from Case 10

From the B.E.C.C. Cytogenetics Unit, Christchurch Hospital, Christchurch, New Zealand. Submitted July 6, 1962; accepted for publication Oct. 8, 1962.

This investigation was supported by the Canterbury and Westland Division, British Empire Cancer Campaign Society.

Blood, Vol. 21, No. 2 (February), 1963
<table>
<thead>
<tr>
<th>Case No.</th>
<th>Sex, Age</th>
<th>Date</th>
<th>Days of Culture</th>
<th>Total Cells</th>
<th>No. with Ph1</th>
<th>% with Ph1</th>
<th>WBC x 10^9</th>
<th>% Immature Cells</th>
<th>Total Cells</th>
<th>No. with Ph1</th>
<th>% with Ph1</th>
<th>M:E Ratio</th>
<th>Nature of Therapy</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F, 49</td>
<td>5/9/61</td>
<td>2</td>
<td>27</td>
<td>16</td>
<td>59</td>
<td>49.2</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>untreated</td>
<td>newly diagnosed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5/8/62</td>
<td>3</td>
<td>58</td>
<td>5</td>
<td>9</td>
<td>8.2</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Myleran</td>
<td>in remission</td>
</tr>
<tr>
<td>2</td>
<td>F, 57</td>
<td>8/30/61</td>
<td>0</td>
<td>35</td>
<td>30</td>
<td>86</td>
<td>510.0</td>
<td>36.2</td>
<td>37</td>
<td>30</td>
<td>81</td>
<td>22:1</td>
<td>untreated</td>
<td>newly diagnosed</td>
</tr>
<tr>
<td>3</td>
<td>M, 49</td>
<td>5/1/61</td>
<td>2</td>
<td>55</td>
<td>0</td>
<td>0</td>
<td>10.4</td>
<td>2.0</td>
<td>38</td>
<td>34</td>
<td>89</td>
<td>18:1</td>
<td>Myleran</td>
<td>in remission</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11/27/61</td>
<td>2</td>
<td>25</td>
<td>7</td>
<td>28</td>
<td>47.9</td>
<td>7.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Myleran</td>
<td>in early relapse</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5/8/62</td>
<td>3</td>
<td>46</td>
<td>13</td>
<td>28</td>
<td>41.7</td>
<td>8.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Myleran</td>
<td>in early relapse</td>
</tr>
<tr>
<td>4</td>
<td>M, 41</td>
<td>3/22/61</td>
<td>50</td>
<td>31</td>
<td>62</td>
<td>8:1</td>
<td>splenic x-ray, Ph</td>
<td>diagnosed 1954, in acute relapse (3/61), complete remission by 6/61 following treatment with 6-MP and prednisone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>6/6/61</td>
<td>50</td>
<td>17</td>
<td>34</td>
<td>1.7:1</td>
<td>Myleran, lately</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7/14/62</td>
<td>3</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>14.0</td>
<td>4.0</td>
<td>37</td>
<td>9</td>
<td>24</td>
<td>3.5:1</td>
<td>Myleran</td>
<td>treated with 6-MP and prednisone</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7/21/61</td>
<td>3</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>7.0</td>
<td>5.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Myleran</td>
<td>prednisone</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8/17/61</td>
<td>3</td>
<td>53</td>
<td>1</td>
<td>2</td>
<td>6.0</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Myleran</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>F, 59</td>
<td>8/30/61</td>
<td>61</td>
<td>61</td>
<td>100</td>
<td>5:1</td>
<td></td>
<td>Myleran diagnosed 1955, in early acute terminal relapse</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>8/29/61</td>
<td>2</td>
<td>34</td>
<td>32</td>
<td>94</td>
<td>54.0</td>
<td>28.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Myleran</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>M, 46</td>
<td>11/28/61</td>
<td>31</td>
<td>11</td>
<td>35</td>
<td>2.5:1</td>
<td></td>
<td>Myleran diagnosed 7/60, in complete remission (no treatment since 5/61)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
|    |    |    |    |    |    |    | splenic x-irradiation and Myleran | diagnosed 2/61, clinically in remission, marrow hyperactive
|----|----|----|----|----|----|----|-----------------------------------|--------------------------------------------------
|    |    |    |    |    |    |    | Myleran                           | diagnosed 12/60, in early relapse following discontinuation of treatment in 8/61
|    |    |    |    |    |    |    | untreated                         | newly diagnosed                                   
|    |    |    |    |    |    |    | splenic x-irradiation and Myleran | diagnosed 7/1/60, in full remission 7/61 when treatment was discontinued early relapse
|    |    |    |    |    |    |    |                                  | newly diagnosed                                   
|    |    |    |    |    |    |    | following splenic irradiation (one course) | diagnosed 9/60, in relapse having defaulted treatment for 6 months

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Total</th>
<th>698  31.8</th>
<th>(average)</th>
<th>526  73.0</th>
<th>(average)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(average)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

From www.bloodjournal.org by guest on August 30, 2017. For personal use only.
was also cultured by the method of Moorhead et al. The direct preparations in Cases 4 and 5 were made by the method of Kinlough et al. Specimen 3/23/61 was obtained 2 hours after the intravenous injection of 6 mg. of Colcemid. Colcemid was not given to this patient on subsequent occasions nor to Patient 5 or any other patient.

The direct method of making marrow preparations was modified in Cases 3, 6, 7, 8, 10, 11 and 12. In these about 1 ml. marrow aspirate was dispersed in 8 ml. ice-cold Hanks solution containing heparin and 0.004 per cent colchicine. The mixture was allowed to stand at room temperature for 1 hour. To give hypotonic treatment, 3 ml. of the cell suspension were mixed with 9 ml. of distilled water and kept for 10–15 minutes, or alternatively 2–3 ml. of the cell suspension were washed in fresh Hanks solution and centrifuged; most of the supernatant was then removed and the remaining 0.5–1 ml. were mixed with 1.5–3 ml. distilled water and left for 10–15 minutes. The solution was again centrifuged, the supernatant discarded and the cells fixed in acetic-alcohol for 30 minutes. Cells were spread by air-drying in all marrows except that of Case 3 in which the squashing technic was used. The preparations were stained by Giemsa or acetic-orcein.

RESULTS

Our findings are summarized in tables 1 and 2. Both in the blood and marrow the modal chromosome number was 46 in every case. Seventy-six and eight-tenths per cent of the mitoses in the blood and 73.4 per cent of those in the marrow were euploid. In the blood there were 14.3 per cent hypoploid, 6.7 per cent hyperploid and 2.2 per cent polyploid figures; in the marrow 23.4 per cent hypoploid, 1.1 per cent hyperploid and 2.1 per cent polyploid figures (74.7 per cent euploid, 21.8 per cent hypoploid, 1.2 per cent hyperploid and 2.2 per cent polyploid if the marrow culture 1/8/62, Case 10, is omitted). Because of the difficulty of tracing the small acrocentric chromosomes in polyploid cells, these are low estimates of the actual numbers of polyploid cells present.

The only constant chromosome abnormality was the Ph1. This was found in all 12 patients. In eight it was present in both blood and marrow, and in one in the marrow but not in the blood (Case 7). In two other patients, no marrow preparations were obtained, but the Ph1 was present in the blood (Cases 1 and 9), and in the last patient the marrow showed the Ph1 but two blood cultures were unsuccessful (Case 6). The percentage of Ph1-positive mitoses varied from 2 to 100. Its relationship to the patients' clinical and hematologic status is discussed below.

Chromosome abnormalities other than the Ph1 were found in a number of patients, as indicated in table 2. All of these had had previous therapy. A minute chromosome replacing a second small acrocentric was found in many mitoses in Case 4; this case has been reported in detail. In Case 5, 72 per cent of the marrow and 85 per cent of the blood cells showed replacement of one of the 6-X-12 group of chromosomes by a small metacentric chromosome; the latter had been lost from most of the cells which contained only 45 chromosomes. In Case 7 there was much chromosome damage, including broken chromosomes and deletions, and the high proportion of hyperploid counts was due to the presence of this chromosome fragmentation. Chromosome damage occurred in cells with and without the Ph1 abnormality. In Case 10 the aneuploid counts appeared to be due to chromosome abnormalities associated with a self-propagating structural interchange.
DISCUSSION

Our findings have confirmed those of Nowell and Hungerford,13-15 Baikie et al.,2 and Tough et al.18 by showing that a high proportion of blood cells in chronic granulocytic leukemia contain the abnormal Ph1 chromosome. None of our cases failed to show this abnormality, either in the blood or marrow or both. Some of the patients were untreated, some in remission, and others in the terminal acute phase; all but one of the blood specimens were cultured, but the marrows were all examined by direct methods. We conclude that the Ph1 is neither produced by therapy given to the patient nor by an artifact caused by abnormal in vitro conditions as suggested by Trujillo et al.,19 but that it is in some way related to the leukemic process itself.

Constancy of Ph1. The 100 per cent incidence of the Ph1 in our patients is higher than that previously reported. Nowell and Hungerford15 found the abnormality in 11 of 12 and Tough et al.18 in 13 of 18 patients. Of these authors' six patients without the Ph1, three were in the acute phase; the other three, all reported by Tough et al.,18 were regarded as atypical, with an unusually long clinical history and a relatively benign course. In all six, studies were made of the blood but not of the marrow, and only single cultures were mentioned.

If we had confined our own attention to the blood, one patient—No. 7—would have been scored as Ph1-negative. Moreover, our patient No. 3 had one, and No. 4 had two negative cultures before the Ph1 was found in the blood, but in all three a high proportion of the mitoses in direct marrow preparations showed the abnormality. Without repeated blood cultures as well as marrow preparations, the incidence of Ph1 in our patients would thus have appeared to be 9 in 12 or 75 per cent. The fact that it was really 100 per cent would indicate that a higher figure might have been found by others working with the same methods as ours.

All our cases were typical of chronic granulocytic leukemia in their hematologic features and clinical course. Less typical cases occur, however, not infrequently, as shown by the material of Tough et al.18 In some such cases, as in a recently reported instance,20 diagnostic difficulties may arise, especially that of their differentiation from myelofibrosis with myeloid metaplasia. When no Ph1 is found, this may thus mean either leukemia without a chromosome abnormality or a mistaken diagnosis. Since there is as yet no unequivocal criterion of what constitutes "leukemia,"20 it may not always be possible to classify such Ph1-negative cases securely. We believe that, in spite of our present results, it would be premature to make a diagnosis of chronic granulocytic leukemia dependent on the finding of the Ph1 abnormality; a normal chromosome constitution would be a strong argument against the diagnosis but would not necessarily invalidate it.

Means of demonstrating Ph1. The proportion of Ph1-positive mitoses was much higher in our direct marrow preparations than in blood cultures (73.0 per cent to 31.8 per cent). Our blood findings are similar to those of Tough et al.18 (34.8 per cent Ph1-positives). No comparable figures have been published for the marrow.
### Table 2.—Chromosome Counts and Abnormalities

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Date</th>
<th>Type of Preparation</th>
<th>Chromosome Counts</th>
<th>Ph(^1) Present</th>
<th>Ph(^1) Absent</th>
<th>Ph(^1) Doubtful</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5/9/61</td>
<td>blood</td>
<td>&lt;43  43  44  45  46  47  48  49  Polyplody  Total</td>
<td>16  10  1</td>
<td>1</td>
<td>previously reported: Fitzgerald(^2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5/8/62</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>8/30/61</td>
<td>marrow</td>
<td>5  1  1  1  27  2</td>
<td>37  30  3  4</td>
<td>2</td>
<td>previously reported: Fitzgerald(^2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8/30/61</td>
<td>blood</td>
<td>2  1  1  3  27  1</td>
<td>35  30  3  2</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>5/1/61</td>
<td>blood</td>
<td>-  -  -  -  53  2</td>
<td>55  49  6</td>
<td>6</td>
<td>previously reported: Fitzgerald(^2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11/27/61</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5/8/62</td>
<td>marrow</td>
<td>3  1  1  2  31  -</td>
<td>38  34  1  5</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5/8/62</td>
<td>blood</td>
<td>-  -  -  -  41  3</td>
<td>46  13  28  5</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>3/22/61</td>
<td>marrow</td>
<td>1  2  2  7  38  -</td>
<td>50  31  19</td>
<td>19</td>
<td>“minute” chromosome</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6/6/61</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>abnormally previously reported: Adams et al.(^1)</td>
</tr>
<tr>
<td></td>
<td>7/14/61</td>
<td>marrow</td>
<td>-  3  2  5  40  -</td>
<td>50  17  33</td>
<td>33</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7/14/61</td>
<td>marrow</td>
<td>-  3  4  29  -</td>
<td>37  9  28</td>
<td>28</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7/14/61</td>
<td>marrow</td>
<td>-  1  1  9  -</td>
<td>11  -  11</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7/21/61</td>
<td>blood</td>
<td>-  -  -  1  11  2</td>
<td>14  -  14</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8/17/61</td>
<td>blood</td>
<td>-  3  1  3  36  6</td>
<td>53  1  52</td>
<td>52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>8/30/61</td>
<td>marrow</td>
<td>5  -  1  15  39  -</td>
<td>61  61  -  61</td>
<td>-</td>
<td>one of the 6-X-12 group chromosomes is represented by a small metacentric chromosome</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8/30/61</td>
<td>blood</td>
<td>5  1  3  5  20  -</td>
<td>34  32  2  -</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Ph = Philadelphia chromosome

\(^2\) Fitzgerald, Adams, and Gunz
<table>
<thead>
<tr>
<th></th>
<th>Date</th>
<th>Type</th>
<th>2</th>
<th>1</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>8</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>11/28/61</td>
<td>marrow</td>
<td>2</td>
<td>1</td>
<td></td>
<td>22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>12/15/61</td>
<td>blood</td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>6</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>4/13/62</td>
<td>marrow</td>
<td>1</td>
<td>1</td>
<td></td>
<td>5</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>10/27/61</td>
<td>blood</td>
<td></td>
<td>3</td>
<td></td>
<td>5</td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>1/18/62</td>
<td>marrow</td>
<td></td>
<td>2</td>
<td></td>
<td>3</td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>5/16/61</td>
<td>blood</td>
<td>1</td>
<td></td>
<td></td>
<td>2</td>
<td>26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>8/10/61</td>
<td>blood</td>
<td></td>
<td>1</td>
<td>24</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>11/3/61</td>
<td>blood</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>9</td>
<td>46</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1/4/62</td>
<td>blood</td>
<td>2</td>
<td>6</td>
<td>5</td>
<td>46</td>
<td>5</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/8/62</td>
<td>marrow</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>17</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/8/62</td>
<td>(culture)</td>
<td>2</td>
<td>1</td>
<td></td>
<td>2</td>
<td>22</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2/6/62</td>
<td>marrow</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>50</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>2/12/62</td>
<td>marrow</td>
<td>4</td>
<td></td>
<td>2</td>
<td>9</td>
<td></td>
<td>3</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>2/16/62</td>
<td>blood</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>25</td>
<td></td>
<td></td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>4/10/62</td>
<td>marrow</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>18</td>
<td></td>
<td></td>
<td>22</td>
</tr>
<tr>
<td>12</td>
<td>5/25/62</td>
<td>marrow</td>
<td>2</td>
<td></td>
<td></td>
<td>4</td>
<td></td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>8/25/62</td>
<td>blood</td>
<td>2</td>
<td></td>
<td></td>
<td>27</td>
<td></td>
<td>1</td>
<td>33</td>
</tr>
</tbody>
</table>

**CML AND THE PHILADELPHIA CHROMOSOME**

For much chromosome damage present, aneuploid counts mostly due to chromosome abnormalities.
In two untreated patients (Cases 2 and 11), equal numbers of Ph'-positive mitoses were found in blood and marrow when simultaneous preparations were made. The same was true for Patient 12 who was in relapse, having had no treatment for some months. In these cases the blood was virtually a suspension of bone marrow cells, and blood and marrow contained equal numbers of immature myeloid cells capable of division. Apparently no advantage was thus gained by making marrow preparations when patients were in full-blown relapse.

The case was different when there were partial or complete remissions (e.g., 5/8/62, Case 3; 7/14/61, Case 4; 1/4–8/62, Case 10: see table 1). Here the proportion of Ph'-positives in the marrow greatly exceeded that in the blood. This appeared to be the result of comparatively few immature myeloid cells being present in the blood, whereas they were numerous in the marrow. Most of the mitoses in these bloods were probably derived not from myeloid cells but either from mononuclears or, more likely, from lymphocytes which had been transformed so as to become capable of division; there is no evidence that leukocytes, other than those belonging to the myeloid series, show the Ph' abnormality, and one would therefore expect to find fewer abnormal mitoses the more closely the blood count approached normal.

Figures 1–3 show the percentages of Ph'-positive cells charted respectively against the total leukocyte count, the percentage of immature cells (myeloblasts and myelocytes) in the blood, and the myeloid:erythroid ratio in the marrow. It can be seen that a positive correlation existed in all three instances, but that it was strongest between the total leukocyte count and the percentage of Ph'-positive mitoses in the blood. This was not unexpected since both the definition of “immature” cells and the calculation of the M:E ratio are subject to larger errors, especially subjective, than the enumeration of leukocytes. The rather wide scatter of the points in figures 1 and 2 was partially explained by the fact that counts were made indiscriminately after 2, 3 or 4 days of culture. It has been shown that a higher proportion of Ph'-positive cells is obtained after 48 than after 72 hours of culture, and in our own two cases in which direct comparisons between successive days could be made, the findings supported those of Nowell and Hungerford.

It appears clear from our results that during remissions of chronic granulocytic leukemia the chances of finding the Ph' are much greater in the marrow than the blood. The more complete the remission, the smaller the proportion of abnormal mitoses in the blood, and complete suppression of all Ph' forms may occur. However we have yet to find a completely negative marrow preparation, and considerable numbers of Ph'-positive mitoses were always present even in marrows which looked otherwise normal, with M:E ratios of 2 or 3:1 (table 1).

Both cases 4 and 5 were in the acute phase when first examined for their chromosome constitution; No. 4 entered a remission following the first mar-

*Correlations were kindly calculated by Miss M. T. K. Chung, M.Sc., Applied Mathematics Laboratory, D.S.I.R., Christchurch.
row examination; No. 5 died shortly after we had studied her. We had no
difficulty in demonstrating the Ph1 in either, in contrast to two of three pa-
tients in the acute phase described by Tough et al.,18 and one by Nowell and
Hungerford,15 in whom no Ph1 was found. Possibly the well-known difficulties
of cultivating leukemic blasts in vitro may account for the discrepancy, and
thus direct marrow preparations may be of particular importance in the acute
phase. Similar views have been expressed by Sandberg et al.16,17

It should be noted that there was no significant difference between blood
cultures and direct marrow preparations in the over-all proportion of aneu-
uploid mitoses (23.2 per cent and 25.3 per cent respectively; table 2). However,
the blood had a lower proportion of hypoploids (14.3 per cent against
21.8 per cent), but more hyperploids, excluding polyploids (6.7 per cent
against 1.2 per cent). Since many hypoploids probably result from loss of
chromosomes during the spreading of films, this suggests a somewhat greater
liability of marrow cells to be damaged during preparation. The greater
degree of hyperploidy in the blood, on the other hand, may signify that culture
itself led to an increase in abnormalities over those initially present in the
myeloid cells.
In contrast to many acute leukemias, the chronic granulocytic form nearly always presents initially with high leukocyte counts. The effect of all kinds of therapy is generally to lower the total counts and the proportion of immature forms in the blood, and also to cause the marrow composition to return towards normal. These effects could be clearly seen in our patients (table 1). Since, as has already been pointed out, relatively normal counts were associated, in both blood and marrow, with relatively low proportions of Ph'-positive mitoses, whereas highly abnormal bloods and marrows had very many such mitoses, the inference appears justified that therapy caused a diminution in the number of Ph'-carrying, and thus probably, leukemic cells.

In the blood the relationship between therapy and the diminution and occasional disappearance of Ph'-positives appears relatively simple: during treatment differential counts show a concurrent disappearance of myeloid cells capable of dividing, and normal monocyte or lymphocyte mitoses rise to take the place of those of abnormal leukemic ones. The events in the marrow, however, pose a more difficult problem. Here, although the proportion and absolute number of immature myeloid cells decreased as a result of treat-
CML AND THE PHILADELPHIA CHROMOSOME

Fig. 3.—Relationship between M:E ratio in marrow and percentage of mitoses showing Ph1 chromosome.

ment, this class of cell does not disappear; concurrently with its diminution there is an increase in erythroid precursors, most of which divide mitotically, probably at a faster rate than do the myeloid cells. Did the diminution of Ph1-positive mitoses in the marrow of our patients mean a replacement of leukemic by non-leukemic myeloid cells, or was it largely the result of the increasing proportion of erythroid mitoses?

Our findings do not enable us to answer this question with certainty, but we believe that the influence of the erythroid mitoses in our preparations was a rather minor one. It seemed probable that the physical nature of erythroblast mitoses was such that it was rarely possible to spread the chromosomes satisfactorily enough for counting. We saw in the direct preparations many very small tightly entangled groups of chromosomes, and these we believe at present to be derived from the numerous small erythroblasts in such marrows. Studies on the more precise identification of mitotic cells are in progress, but in the meantime it is our impression that most of the countable mitoses in marrow were derived from myeloid cells.

If this was so, then the diminution in Ph1-positive mitoses under treatment indicated a partial replacement of leukemic by normal myeloid cells. The

*Erythroid cells are presumed not to carry the Ph1 abnormality, but we are not aware of any direct evidence to this effect.
replacement has never yet, however, been seen to be complete, and thus it
must be concluded that leukemic cells were suppressed but not utterly
destroyed; a conclusion in full accord with all that is otherwise known of
the mode of action of therapeutic agents in this form of leukemia.

The significance of the Ph' chromosome. In our material, the Ph' chromo-
some was a constant accompaniment of chronic granulocytic leukemia. Other
abnormalities, mentioned briefly above, were inconstant from case to case
and may well have resulted from treatment, as was suggested in correspond-
ing cases by Tough et al. and by Nowell and Hungerford. They were
nevertheless of some theoretical interest and will be the subject of a further
communication.

As stated by Nowell and Hungerford, the Ph' chromosome demonstrates
"a specific, consistent chromosome change associated with a particular human
neoplasm . . . Such an association has not yet been demonstrated in any other
human tumor." The Ph' chromosome would appear to characterize a specific
line of malignant cells; a view which is supported by our finding of positive
correlations between the percentage of cells carrying Ph' on the one hand,
and the total leukocyte count, the percentage of immature cells, and the M:E
ratio on the other. The most important question yet to be solved is whether
Ph' is indeed, as further suggested by Nowell and Hungerford, "the chrom-
osome change which confers on the leukocytes their neoplastic character."

Although this conclusion is an immediately attractive one, there is as yet
no evidence to prove it. It is also possible to assume, on the contrary, that
rather than causing the neoplastic change, the chromosome abnormality arises
as its result, the neoplastic environment favoring, for reasons yet unknown,
the development of an abnormal clone with the particular Ph' constitution.

The Ph' is present only in blood cells, and probably only in those of the
myeloid series. It is not found in skin cultures and thus the presumption is
that the abnormality is an acquired one confined to a single cell series in
the hematopoietic organs. This state of affairs may be compared with that in
some cases of "familial" chronic lymphocytic leukemia recently described by
us. Here an abnormality of a small acrocentric, termed Ch', was found in
100 per cent of the blood cells of two siblings with chronic lymphocytic leu-
kemia and in several of their relatives who did not have clinical leukemia.
The Ch' abnormality was thus inherited and definitely preceded the onset
of leukemia to which, presumably, it predisposed those carrying it. The Ph' abnor-
mality, by contrast, is not inherited, and there has been so far no direct
evidence to show that it is present before the onset of clinical leukemia. As
yet, the question of the precise relationship of the Ph' chromosome to the
leukemic process must remain open.

SUMMARY

Twelve patients with chronic granulocytic leukemia were examined for
the presence of the Ph' chromosome abnormality at various stages of their
disease. The abnormality was demonstrated either in the blood or marrow of
all patients. Patients in relapse showed very high numbers of Ph'-positive
mitoses in both blood and marrow; those in remission showed relatively few
or no positives in the blood but sizeable numbers in the marrow. There was a positive correlation between the degrees of abnormality of blood and marrow on the one hand, and the percentage of Ph'-positive mitoses on the other. Direct examination of the marrow for the demonstration of the Ph' appeared of particular value in the acute phase of chronic granulocytic leukemia.

It seemed likely that therapy depressed the number of abnormal (Ph'-positive) mitoses. There was, however, no evidence that these ever disappeared from the marrow, even in complete remission.

The findings presented and those in the literature did not make it possible to decide in what way the Ph' chromosome abnormality is related to the onset of leukemia.

**SUMMARIO IN INTERLINGUA**

Decé-duo patientes con chronic leucemia granulocytic esseva examine con respecto al presentia del anormalitate de chromosomas Ph' a varie studios in le curso de lor maladia. Le anormalitate esseva demonstrate in le sanguine o alora in le medulla del patientes. Patientes in recidiva monstrava altissime numeros de mitoses a Ph' tanto in le sanguine como etiam in le medulla. Patientes in remission monstrava relativemente pauc tales in le sanguine sed considerabile numeros in le medulla. Esseva constatate un correlation positive inter le grados de anormalitate del sanguine e del medulla de un latere e le procentage de mitoses a Ph' del altere. Le directe examine del medulla pro le demonstration de Ph' pareva de valor particular in le phase acute de chronic leucemia granulocytic.

Il pareva probable que le therapia deprimeva le numero del mitoses anormal (a Ph'). Esseva notate, nonobstante, nulle evidentia que istos unquam dispareva completemente ab le medulla, non mesmo in remission complete.

Le constatationes hic presentate e le datos trovate in le litteratura non permette decider in qual maniera le anormalitate del chromosoma Ph' es relationate con le declaration de leucemia.

**REFERENCES**

9. Kinlough, M. A., Robson, H. N., and


P. H. Fitzgerald, M.Sc., Research Associate, Cytogenetics Unit, Christchurch Hospital, Christchurch, New Zealand.

Angela Adams, B.Sc., Research Fellow, Cytogenetics Unit, Christchurch Hospital, Christchurch, New Zealand.

Frederick W. Gunz, M.D., Ph.D., Hematologist, North Canterbury Hospital Board, Cytogenetics Unit, Christchurch Hospital, Christchurch, New Zealand.
Chronic Granulocytic Leukemia and the Philadelphia Chromosome

P. H. FITZGERALD, ANGELA ADAMS and FREDERICK W. GUNZ