Chromosomal Dichotomy in Blood and Marrow of Acute Leukemia*

AVERY A. SANDBERG, T. ISHIHARA, L. H. CROSSWHITE, AND THEODORE S. HAUSCHKA

(Departments of Medicine C and Experimental Biology, Roswell Park Memorial Institute and the Medical Foundation of Buffalo, Buffalo, New York)

SUMMARY

The chromosome patterns in freshly aspirated, noncultured marrow of six patients with acute leukemia differed consistently from the findings in cultured blood of the same individuals. Unique anomalies in chromosome number and/or morphology characterized all six marrows but none of the essentially diploid blood cultures. The cytogenetic peculiarities of the leukemic marrows were, on the whole, unrelated to prior therapy or duration of disease. However, in one patient, beneficial chemotherapy eliminated most of the pathological karyotypes from the marrow, and this was accompanied by reappearance of diploid mitoses and resumption of histologically almost normal hemopoiesis. Apparent aneuploidy in the blood cultures averaged $13.2 \pm 1.7$ per cent, and the mean frequency of abnormal metaphases in the marrows was $94.1 \pm 0.9$ per cent. Because of their pronounced modality and individuality, the chromosome abnormalities are interpreted as belonging to the leukemic stem cells that propagate the disease in vivo. Proliferation of these malignant cells was handicapped in vitro by a longer lag-phase, a trend toward maturation, or a metabolic disadvantage. Cell division in the blood cultures was limited to presumably nonmalignant, hemopoietic elements with the normal diploid human karyotype. The blood culture method appears inadequate for detecting the propagative karyotypes in acute leukemia.

The search for chromosome changes in human leukemia has been approached by three methods: short-term marrow culture, originated by Ford, Jacobs, and Lajtha (8); blood culture, initiated by Nowell, Hungerford, and Brooks (19); and “direct” analysis of freshly aspirated marrow, after colcemide injection (4, 14, 15) or without any pretreatment of the patient (11, 21, 22). In the present report, we are not concerned with the academic question of the primary leukemic karyotype as an etiologic factor. This possibility lies beyond present experimental reach. The microscope can, however, resolve the chromosomes in the propagative “stem cells” that maintain the leukemic state in the patient, and must be dealt with clinically. Do the modal nuclei of acute leukemia differ from the diploid norm in control marrows (21), and, if so, how and in what relation to histology, treatment, and duration of disease?

Acute leukemia is a proliferative disease of the marrow. Examination of chromosome patterns immediately after marrow aspiration is, therefore, the most direct approach to the leukemic nucleus. However, difficulties of coordination between clinic and cytology laboratory and reliance on the convenient short-term culture technics have postponed critical comparison of the in vitro methods with “direct” analysis. Contradictions in the chromosome data for acute leukemia are evident from Table 1. Chromosome counts in the “direct” marrow aspirates of five patients with acute and three with chronic leukemia—all pretreated with colcemide—led Bayreuther (4), and personal communication to regard human leukemias as diploid neoplasms typified by “the species-specific and sex-specific normal chromosome complement.”

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was not generally confirmed at other laboratories (1-3, 5-8, 10, 11, 14, 18, 21, 22), where attention was paid not merely to numerical aneuploidy but to structural pseudo-diploidy.

The investigations of Ford and his collaborators (6-9), like those of Baikie et al. (2, 3), were based largely on cultures of leukemic marrow and indicate a rather high incidence of karyotypic anomalies in both acute and chronic cases. Nowell and Hungerford (18), relying on the blood culture technic, discovered the minute Ph₁ chromosome which is now regarded as almost diagnostic for chronic myelocytic leukemia. However, the same preparations?—In an earlier patient series we included statistical comparison of abnormal karyotypes in marrow culture (Table 1, line 5) and analysis of marrow metaphases examined directly, without recourse to colchicine or incubation (Table 1, line 7). The number of nuclear anomalies found in the “direct” marrow examination decreased significantly when the same marrows were incubated for 10–12 hours. The chromosomes of acute leukemia have been investigated for the most part in dividing cells of the blood or the marrow. In the present series of six patients, we have compared the metaphases in cultured blood

### TABLE 1

**CHROMOSOME NUMBERS IN 71 ACUTE LEUKEMIAS OF MAN, AS DETERMINED BY THREE METHODS:**

<table>
<thead>
<tr>
<th>No. cases</th>
<th>Method</th>
<th>Modal chromosome number</th>
<th>Total exact counts</th>
<th>Per cent aneuploid metaphases</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Blood culture</td>
<td>3</td>
<td>37</td>
<td>8</td>
</tr>
<tr>
<td>7</td>
<td>“ ”</td>
<td>7</td>
<td>401</td>
<td>10</td>
</tr>
<tr>
<td>13*</td>
<td>“ ”</td>
<td>11</td>
<td>2</td>
<td>502</td>
</tr>
<tr>
<td>15</td>
<td>Marrow culture</td>
<td>10</td>
<td>5</td>
<td>826</td>
</tr>
<tr>
<td>5</td>
<td>“ ”</td>
<td>5</td>
<td>49</td>
<td>39</td>
</tr>
<tr>
<td>8</td>
<td>“ ”</td>
<td>6</td>
<td>2</td>
<td>472</td>
</tr>
<tr>
<td>1</td>
<td>“ ”</td>
<td>1</td>
<td>1</td>
<td>200†</td>
</tr>
</tbody>
</table>

* In seven of these thirteen patients marrow cultures were also studied (see line 4 of this table).
† According to Bayreuther (4), the diploid metaphases all “revealed the species-specific and sex-specific normal chromosome complement.” The aneuploid value of 21 per cent was given to us in a personal communication in which it was stated that all five patients were pretreated with colcemide.
‡ Of these 900 cells, 165 had 51–53 chromosomes.

workers (13, 17) reported less than 10 per cent random chromosome variation in the otherwise diploid blood cultures from nine patients with acute leukemia. The finding of diploidy in these blood cultures agrees at least superficially with the diploid or pseudo-diploid modes predominating in the noncultured marrows of ten previously reported patients with acute myeloblastic leukemia (21). On the other hand, eight of eleven acute lymphoblastic marrows had morphologically individualized aneuploid modes (21, 22). The histology of acute leukemia may constitute one source of discrepancy in published data. However, the major confusion arises from divergent interpretation of culture data and “direct” marrow findings.

Which are the neoplastic metaphases in these and noncultured marrow from the same individuals. Our aim was to evaluate blood culture as a cytological assay method for acute leukemia and to arrive at criteria for the identification of leukemic karyotypes.

### METHODS AND CASE REPORTS

Marrow preparation by our “direct” squash technic (21, 22) involves the following essential steps: Aspiration of 1–2 ml. of sternal or iliac crest marrow under local anesthesia; suspension of the aspirate in 20 ml. of cold isotonic Earle solution; 1:4 dilution of this suspension with 0.44 per cent sodium citrate; swelling of the cells held for 15 minutes in this hypotonic medium; light packing of the marrow cells by slow centrifugation, followed by decanting the supernatant fluid; fixation
of the packed cell plug in 50 per cent glacial acetic acid or acetic alcohol for 30 minutes; decanting of fixative and addition of 1 ml. of filtered 2 per cent orcein in 65 per cent acetic acid; uniform suspension of the cells in the stain for 10 minutes by gentle agitation with a Pasteur pipette; squashing by thumb pressure of a drop of stained cells mounted free of air bubbles between a scrubulously lint-free slide and a #2 rectangular coverslip; air-sealing the edges of the coverslip with heated beeswax or a mixture of paraffin and vaseline.

The leukemic blood cultures and cytological preparations therefrom were made according to the procedure of Moorhead et al. (16). Phytohemagglutinin M (Difco), the original mucoprotein, was used for leukocyte separation from whole blood. A drop of phytohemagglutinin P (Difco), the purified globulin, was added to each culture vial before incubation. Dr. P. C. Nowell (personal communication) established lack of mitogenic activity in the M-form and recommended routine addition of the strongly mitogenic P-fraction to the medium. The leukemic blood samples in the present series were obtained at the time of marrow aspiration; they were then incubated for exactly 48 hours, since shorter or longer periods in vitro resulted in a reduced mitotic index. Colchicine was added to the blood cultures during the last hour of incubation.

Well spread metaphases in the "direct" marrow fixations (540 exact counts) and cultured blood preparations (395 exact counts) from six patients with acute leukemia were counted under oil immersion at a magnification of 970 X. Photomicrographs of numerous countable plates were re-examined, and 189 karyotypes (like Fig. 6) were prepared by matching the chromosomes cut out of enlarged prints and pasting them on sheets of paper according to size and probable homology. This tedious procedure is essential for detailed study of the more subtle chromosomal aberrations, such as possible trisomy, monosomy, and translocations, especially in pseudo-diploid metaphases. For the identification of homologous chromosomes, the Denver number system (12) was combined with Patau's alphabetical grouping (20). Although it is possible to classify chromosomes on the basis of their relative lengths and centromere positions, the arbitrary labeling of an individual "leukemic" chromosome with a group symbol does not certify its normal homology.

During November and December, 1961, we saw ten patients (not previously reported) with untreated acute leukemia or in relapse of the disease following various courses of therapy. Satisfactory "direct" marrow fixations and blood cultures were available for comparative study from only six patients. This group is essentially unselected, except for technical success with both their marrow aspirates and their blood cultures:

**Patient #1**

**Patient.**—6 years old, white male. Acute myeloblastic leukemia diagnosed 7-15-61; died 12-27-61.

**Therapy.**—6-mercaptopurine 7-15-61 to 9-29-61; prednisone 7-15-61 to 8-10-61; radiation (690 rads) to upper thoracic region 7-19-61 to 7-31-61; methylglyoxal-bis-guanylhydrazone 10-3-61 to 11-17-61; radiation (600 rads) to temporal areas 10-16-61 to 10-23-61.

Blood and marrow findings at time (11-22-61) of chromosome analysis:

**Blood.**—Hemoglobin, 9.0 gm. per cent; platelets, 15,000/cu mm; white blood cell count and differential: 1550/cu mm (20 per cent neutrophils, 54 per cent lymphocytes, 6 per cent monocytes, 2 per cent eosinophils, 4 per cent myelocytes, 14 per cent myeloblasts).

**Marrow.**—Very cellular (63 per cent myeloblasts, 10 per cent promyelocytes, 16 per cent myelocytes, 7 per cent neutrophils, 3 per cent lymphocytes, 1 per cent normoblasts, no megakaryocytes). Some of the myeloblasts contained large Auer bodies.

**Patient #2**


**Therapy.**—None for the leukemia; however, this patient underwent a right mastectomy for carcinoma on 7-20-61, followed by a brief course of 5-fluorouracil, 870 mg. daily for 4 days, starting on 7-28-61.

Blood and marrow findings at time (11-21-61) of chromosome analysis:

**Blood.**—Hemoglobin, 10.6 gm. per cent; platelets, 7,500/cu mm; white blood cell count and differential: 16-61 to 10-23-61.

**Marrow.**—Very cellular (84 per cent myeloblasts, 3 per cent promyelocytes, 6 per cent myelocytes, 4 per cent lymphocytes, 2 per cent neutrophils, occasional normoblast, no megakaryocytes). Some of the myeloblasts contained Auer bodies.

**Patient #3**

**Patient.**—5 years old, white female. Acute lymphoblastic leukemia diagnosed 6-25-60; living as of 1-19-62.
Therapy.—Prednisone 6-25-60 to 7-1-60; 6-mercaptopurine 6-25-60 to 6-20-61; methotrexate 6-20-61 to 6-29-61 and 7-6-61 to 11-6-61; prednisone 6-29-61 to 8-17-61; leurocristine 11-10-61 to 11-24-61.

Blood and marrow findings at times (11-9-61 and 11-24-61) of chromosome analysis:

Blood.—(11-9-61) Hemoglobin, 12 gm. percent; platelets, 25,000/cu mm; white blood cell count and differential: 4800/cu mm (14 per cent neutrophils, 4 per cent nonsegmented neutrophils, 60 per cent lymphocytes, 2 per cent eosinophils, 1 per cent monocytes, 19 per cent lymphoblasts).

Marrow.—(11-9-61) Very cellular (98 per cent lymphoblasts, 1.4 per cent normoblasts, no megakaryocytes).

Blood.—(11-24-61) Hemoglobin, 7.2 gm. per cent; platelets, 7500/cu mm; white blood cell count and differential: 1750/cu mm (16 per cent neutrophils, 10 per cent nonsegmented neutrophils, 74 per cent lymphocytes).

Marrow.—(11-24-61) Moderately hypocellular (41 per cent lymphoblasts, 16 per cent lymphocytes, 28 per cent normoblasts, 15 per cent granulocytes, no megakaryocytes).

Patient #4

Patient.—4 years old, white male. Acute lymphoblastic leukemia diagnosed 8-14-60; living as of 1-19-62.

Therapy.—Prednisone 8-20-60 to 9-20-60; methotrexate 8-20-60 to 7-27-61; prednisone 7-27-61 to 8-29-61; 6-mercaptopurine 7-8-61 to 11-14-61; leurocristine 11-21-61, 11-27-61, 12-8-61, 12-15-61, 12-22-61, 12-29-61, 1-5-62.

Blood and marrow findings at times (11-17-61, 12-15-61, and 1-12-62) of chromosome analysis:

Blood.—(11-17-61) Hemoglobin, 13.4 gm. per cent; platelets, 85,000/cu mm.; white blood cell count and differential: 4100/cu mm (3 per cent neutrophils, 2 per cent eosinophils, 2 per cent monocytes, 78 per cent lymphocytes, 15 per cent lymphoblasts).

Marrow.—(11-17-61) Very cellular (90 per cent lymphoblasts, 3 per cent lymphocytes, 7 per cent normoblasts, no megakaryocytes).

Marrow.—(12-15-61) Normal cellularity (M:E reversed owing to >80 per cent normoblasts, only 5 per cent lymphoblasts, 3 per cent lymphocytes, 11 per cent granulocytic cells, normal number of megakaryocytes).

Marrow.—(1-12-62) Very cellular (M:E reversed owing to a large number of nucleated red blood cells, 2 per cent myeloblasts, 3 per cent promyelocytes, 11 per cent myelocytes, 9 per cent mature granulocytes, 6 per cent lymphocytes, 5 per cent pronormoblasts, 64 per cent normoblasts, normal number of megakaryocytes).

RESULTS

Chromosome study was attempted in ten patients with acute leukemia. Suitable marrow aspirates and blood cultures were obtained from six cases; one subject yielded only marrow, two individuals only blood, and in one patient both methods failed. The modal karyotypes were numerically and/or structurally abnormal in the "direct" marrow samples of all seven patients whose aspirates were sufficiently cellular for cytologic examination. In striking contrast, all of the
eight blood cultures had chromosome number modes at 46, with normal karyotypes. This chromosomal dichotomy in freshly fixed noncultured marrow and incubated blood was analyzed in considerable detail in the six patients from whom we obtained both types of specimens.

Table 2 summarizes the findings for these six cases with respect to chromosome number distribution, frequency of the prominent structural anomalies, and—in one patient—reversion of the aneuploid marrow toward diploidy during beneficial chemotherapy. On "direct" inspection, the six initial marrow samples had aneuploid modes of 45, 45, 94, 51, 47, and pseudo-diploid 46, respectively. Among these leukemic marrows, four patient #1 had 45 chromosomes. Karyotype analysis in 22 cells revealed that two chromosomes were regularly missing from Group C. Each of the pairs D15, E16, and F20 contained one extra unit. Nearly all the modal metaphases had only two small acrocentrics in Group G21–22, instead of the usual four (Fig. 1). The dividing cells in the blood culture of this patient had a 72 per cent mode at 46, without discernible morphologic departures from the norm.

The modal chromosome number of patient #2 was 45, comprising 68 per cent of the metaphases counted. A prominent ring chromosome was present in 58 per cent of the total scored (Fig. 3), being doubled in the few tetraploid metaphases encountered (Fig. 4). Other peculiarities of this leukemic marrow included: a small, extra unit in the category of Group G (frequency, 55 per cent); reduction of Group C from the usual fourteen to only twelve; monosomy in Groups D and E; and trisomy in Group A. Findings in the corresponding blood culture were again indistinguishable from the normal.

The cell population profile in the marrow of patient #3 was bi-modal, more than two-thirds of the cells being in the tetraploid range (principal mode at 94), with the remainder in the diploid region. The hypertetraploid metaphases contained no obvious marker chromosomes. Figure 5 shows one of the marrow metaphases in the sub-tetraploid category. The cells dividing in the blood culture from this patient were 92 per cent diploid; were further characterized by abnormal marker chromosomes (Table 2, last column). The distinguishing features were usually limited to metaphases of the cell-type at and near the mode. Metaphases in the corresponding blood cultures were predominantly diploid; none of them contained the markers seen in the marrows of the same four patients. The over-all incidence of aneuploidy or pseudo-diploidy in the six initial marrow samplings was 94.1 ± 0.9 per cent. In the cultured blood specimens obtained at the same time, random numerical variation amounted to only 13.2 ± 1.7 per cent. The physiological basis of this consistent and significant difference (P < 0.0001) is discussed below.

Seventy per cent of the marrow mitoses of

### Table 2

**Comparison of Chromosome Patterns Revealed by the "Direct" Marrow Method and by Blood Culture in Acute Leukemia**

<table>
<thead>
<tr>
<th>Case Number, Sex, Age, Type of Leukemia, Treatment Status</th>
<th>Source of Cells</th>
<th>Total Count</th>
<th>Chromosome Number</th>
<th>Markers Chromosomes (Per Cent Frequency)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1, m, 8 yr., AML, treated</td>
<td>Marrow</td>
<td>85</td>
<td>&lt;44 45 46 47 48 49 50 51</td>
<td>52 54 55 56 57 58 59 60 61 62</td>
</tr>
<tr>
<td></td>
<td>Blood</td>
<td>100</td>
<td>1 5 10 75 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100</td>
<td></td>
</tr>
<tr>
<td>Case 2, f, 62 yr., AML, untreated</td>
<td>Marrow</td>
<td>65</td>
<td>10 13 41 1 1</td>
<td>5 7 31</td>
</tr>
<tr>
<td></td>
<td>Blood</td>
<td>50</td>
<td>1 2 46 1</td>
<td></td>
</tr>
<tr>
<td>Case 3, f, 5 yr., ALL, treated</td>
<td>Marrow</td>
<td>70</td>
<td>9 3 10 9 5 7 16 96 6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Blood</td>
<td>50</td>
<td>1 2 46 1</td>
<td></td>
</tr>
<tr>
<td>Case 4, m, 4 yr., ALL, treated</td>
<td>Marrow</td>
<td>100</td>
<td>3 1 7 16 96 6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Blood</td>
<td>82</td>
<td>2 23 2</td>
<td></td>
</tr>
<tr>
<td>Case 5, m, 9 yr., ALL, untreated</td>
<td>Marrow</td>
<td>75</td>
<td>1 1 8 6 5 5 8 1 14 27 15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Blood</td>
<td>55</td>
<td>1 2 51 1</td>
<td></td>
</tr>
<tr>
<td>Case 6, f, 43 yr.</td>
<td>Marrow</td>
<td>150</td>
<td>5 8 151 6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Blood</td>
<td>85</td>
<td>1 2 5 76 1</td>
<td></td>
</tr>
</tbody>
</table>

* In each case the first marrow and blood sample was obtained on the same day. Second blood sample taken 2 weeks after first sampling. Second and third marrow aspiration performed 4 and 8 weeks after first sampling shows response to chemotherapy.

† The large metacentric marker was seen only in hyperdiploid metaphases.

Symbols: AML = acute myeloblastic leukemia; ALL = acute lymphoblastic leukemia.
not a single polyploid division was found in the blood. Two weeks later, after a course of leu-
cristine therapy, the modal chromosome constitution of the marrow was re-examined with atten-
tion to ploidy classes rather than exact numbers. A decided downward shift of chromosome constitution had occurred: about two-thirds of the cells were now classified as near-diploid; the formerly predominant tetraploid population peak had shrunk to one-third, whereas the blood remained almost entirely diploid, as before.

In the first marrow aspirate of patient #4, 74 per cent of the metaphases had a mode of 51 chromosomes. The five excess units in this modal karyotype include a prominent metacentric somewhat larger than pair A1 (Fig. 6, lower right; Figs. 8, 10, and 11, arrows). Four weeks later, following a course of leucristine chemotherapy, the patient's marrow, which earlier had been characterized by a 90 per cent lymphoblast count, contained 80 per cent normoblasts and only 5 per cent lymphoblasts. This dramatic reversal is accompanied by a relatively normal hemopoietic histology coincided with a shift of the chromosome constitution toward a diploid mode (Figs. 7, 9, 11 left), which was nearly complete after 4 weeks of leucristine treatment. Simultaneously with this promising numerical change and the much improved clinical status of the patient, the frequency of cells containing the large metacentric marker dropped from 93 per cent to 9 per cent. Details of these changes may be seen in Table 2, lines 8–11.

The diploid metaphases which appeared increasingly in the marrow of patient #4 during his clinical improvement had structurally normal sets of 46 chromosomes with thin, well delineated chromatids (Figs. 7, 9, 11 left). The normal karyotype of these cells leads us to regard them as non-
leukemic. On the other hand, all the abnormal cells with 51 chromosomes containing the meta-
centric marker had swollen chromatids somewhat fuzzy in outline, grossly distinguishable from the diploid plates even without recourse to counting (Figs. 8, 10, 11 right). This remarkable difference in over-all chromosome appearance may be added to the numerical and structural features whereby occasionally the leukemic elements in a marrow are set apart from the normal. The variation in chromosomal compactness suggests an inherent peculiarity of the leukemic chromatin—perhaps related to the differential chemotherapeutic response—which had its parallel in the improved marrow histology. None of these intriguing observations could have been made in the monoto-
nously diploid blood culture metaphases of patient #4.

In the marrow of patient #5, 84 per cent of the dividing cells had 47 chromosomes. The extra unit was an abnormal submetacentric, smaller than the shortest pair in Group C but larger than E16. This marker resembles a unit seen in the marrows of five other cases of acute leukemia (3, 6, 14; Sandberg et al., unpublished) and in our case #24 with the terminal “blastic phase” of chronic myelocytic leukemia (21). As usual, the marker was absent from the 89 per cent normal diploid blood culture metaphases of patient #5.

The marrow mitoses of patient #6 has a pseudo-
diploid mode of 46. They were typified by a satel-
leted acrocentric much larger than the Y but smaller than D15 (Fig. 12, arrow). The often prominent satellites on this marker preclude our labeling it as an abnormally large Y. It was also much too long to suggest homology with Group G. The blood culture karyotypes were those of a normal male, and none of them contained the unique acrocentric seen in the pseudo-diploid leukemic marrow.

The abnormal karyological idiosyncrasy of each leukaemic marrow, when considered against the normal diploidy of the dividing blood elements, justifies the following general conclusions: The aberrant modal karyotypes in the marrow aspirates represent the stem cells through which each leukemia propagates itself in vitro. Although the blood specimens contain many blast cells, the blasts seem to have a mitotic disadvantage in vitro. The metaphases seen in blood cultures after 48 hours of incubation are predominantly, if not entirely, those of nonmalignant monocytes and large lymphocytes (as in the case of nonleukemic blood). The generative karyotypes of acute leu-
keemia are not demonstrable by the blood culture method.

**DISCUSSION**

Acute leukemia of man is a proliferative disease of the marrow, manifested by over-production of immature, physiologically abnormal leukocytes, varying numbers of which find their way into the bloodstream. Not infrequently, acute (“aleu-
kemic”) leukemia can be diagnosed only in marrow, not from the peripheral blood picture. On a mor-
phological basis, many acute leukaemias can be labeled as lymphoblastic or myeloblastic. Some cases defy precise classification (e.g., “stem cell” leukemia). Even the histologically well defined leukaemias may constitute heterogeneous disease entities differing in their etiology. Therefore, nu-
clear studies aimed at the generative chromosome patterns of acute leukemia are best conducted in specimens of marrow, where the leukemic cells originate and proliferate.
In the leukemic marrow, diploid mitoses of normal blood elements continue their erythropoietic and myelopoietic task side by side with neoplastic stem-cell divisions. Is it possible to identify the malignant metaphases? An obvious limitation of cytogenetic analysis by the acetic orcein method is that it ruptures the cells in order to flatten the metaphase chromosomes into plain view. The histologic derivation of these mitotic cells is undeterminable, since the familiar features of nuclear shape, cytoplasmic differentiation, and nucleocytoplasmic ratio are lacking. Diploid metaphases could therefore be either normal or malignant. Aneuploidy as such does not signify neoplasia; it has been observed in 60 normal control marrows with a frequency of 12.2 ± 0.8 per cent (21). Thus, a squashed metaphase of leukemic marrow: "... there was difficulty in getting enough cells to enter mitosis in the suspensions established from leukemic patients, and sufficient observations to be useful were obtained only from 3 out of the first 12 that were examined."

Tissue-culture assays of the varied cell types composing any neoplasm are subject to the risk of differential mitotic initiation, if not selective overgrowth. Therefore, a chromosome survey conducted in vitro cannot serve as the sole basis for speculations about the leukemic karyotypes that propagate the disease in the patient. The possible bias in such interpretations has been pointed out by Ford and Mole (9) and others (3, 10, 11, 21, 22). Meighan and Stich (15) recommended direct examination of cells "as a general rule" in preference to second-hand information gained from the mitoses in tissue culture.

The marrow is not only the site of origin of acute leukemia but also the site of progressive selection for the neoplastic growth advantage over normal hemopoietic proliferation. Hence, extrapolation of marrow or blood into an artificial environment could abrogate mitotic advantages gained in vivo and initiate re-selection through a new sequence of metabolic adaptations. This dilemma applies even to short-term 48-hour incubation during which some of the cultured cells pass through only one or two mitotic cycles, while many cells fail to divide. No appreciable population shift by selective overgrowth can occur under these in vitro conditions. "What matters here is: Which are the cells that have a mitotic head-start and, therefore, are in metaphase at the moment of fixation for chromosome study?" (10)
An awareness of the physiologic variables that underlie the present comparison of cell divisions in cultured blood and in fresh marrow seems important if we wish to define criteria for recognizing the propagative karyotypes of acute leukemia:

**Cultured leukemic blood.**—The blood specimens drawn from our patients contained many leukemic blast cells and often fewer than 10 per cent of potentially mitotic normal hemic elements, such as monocytes and large lymphocytes. This highly skewed population was incubated for 48 hours in a medium containing Difco 199 nutrient, pooled normal human serum, antibiotics, and phytohemagglutinin P. Colchicine was added during the last hour of incubation. Should one assume *a priori* (13) that the cells arrested in metaphase are, for the most part, derived from the leukemic blasts? It is highly suggestive that longer incubation leads to the disappearance of all mitoses accompanied by maturation of blast cells, as also observed by Nowell (17). Most of the leukemic blasts may, in fact, never have divided. Are the metaphases encountered those of nonmalignant cell divisions? The selective growth advantages gained by the leukemic elements in the patient’s marrow may be neutralized, or turned to a disadvantage, *in vitro*. Among hypothetical growth stimulants operative *in vivo* but absent in blood culture are: growth-enhancing levels of auto-antibody, humoral regulators of hemopoiesis, and an abundance of intermediary metabolites supplied by normal tissue. Further, in treated subjects the leukemic blasts have perhaps been differentially injured by chemotherapy, and this jeopardizes their division potential in culture.

**Direct marrow aspirates.**—In marrow fixed by our direct method, the cells caught in metaphase re Reflect the status quo in the patient’s hemopoietic system. The pronounced modes of aneuploid or pseudo-diploid cells with typical markers, found in the present series of six patients and in direct marrow studies of twelve other individuals with acute leukemia (5, 14, 21), cannot simply be the erratic byproducts of misdivision or mutagenic therapy. The modal concentration and uniformity of these altered cells implicate them in the leukemic process. Whether they are mere epiphenomena (4, 13) of progression toward greater malignancy or constitute the direct descent of a similarly altered ancestral karyotype is irrelevant for the present argument.

We are not concerned with the hypothetical primary chromosome patterns of leukemogenesis but with the real chromosomal dichotomy in fresh leukemic marrow vs. cultured blood. Although we were able to confirm the predominance of diploid mitoses in cultured blood from acute leukemias (13, 17), our aneuploid marrow data invalidate the interpretation of the dividing cells in corresponding blood as leukemic blasts. Whatever the physiologic basis for this glaring difference—diploidy in the blood vs. consistent abnormalities in the marrow—the diploid mitoses in the blood cultures appear to be nonmalignant, and those in the “direct” marrow specimens represent the generative leukemic karyotypes.

Hungerford’s (13) supposition that therapy might account for many of the observed departures from diploidy prompted us to tabulate chromosome data from “direct” marrow examinations in 38 acute leukemias (Table 3) according to whether the patients were treated (T) or not (U). No obvious enhancement of nuclear anomalies due to treatment is discernible. On the contrary, the untreated acute leukemias tend to be karyotypically more abnormal. Duration of disease also was not correlated with nuclear variability. Most of the eighteen untreated leukemias were of recent origin, since therapeutic measures are usually taken soon after diagnosis. Yet ten of these early cases already had individualistic aneuploid modes and only eight were diploid or pseudo-diploid. Among the twenty treated cases some were of relatively long duration, eleven were classified as “diploid,” and nine as aneuploid.

It has been our experience, based on leukemic patients whose marrows were examined before and during chemotherapy (21), and unpublished data), that unsuccessful therapy produced no detectable shifts in the previously established abnormal karyotypic mode. However, ameliorative therapy, when followed by clinical remission, resulted in a partial or complete disappearance of aneuploid modal stem-cell metaphases and a resumption of presumably nonleukemic mitoses with 46 chromosomes. An example of this trend toward normality is case 4 (Table 2, lines 8–10). The parallel between positive clinical response and diploidization of a formerly aneuploid leukemic marrow raises hopes for selective inhibition of leukemic growth by at least some antimetabolites. Certain karyotypes may be more manageable by chemotherapy than others. It is noteworthy that the only two near-tetraploid cases of acute leukemia on record responded well to treatment and survived for exceptionally long periods: Patient #8 in the present series is living and in remission 13 years after diagnosis; patient #8 in our earlier series (21) died after a 5-year see-saw battle with his hyper-tetraploid acute lymphoblastic leukemia.

No chromosome abnormality specific for acute leukemia is yet apparent. Each marrow has its
own peculiar cytogenetic profile, which accounts for the wide range in the pathophysiology and drug response of histologically similar cases. The important discovery of the Ph1 minute chromosome, specific for chronic myelocytic leukemia, was made by Nowell and Hungerford (18) in cultures of peripheral blood. It is intriguing that the pseudo-diploid cells with the Ph1 marker of chronic myelocytic leukemia are able to proliferate in incubated blood, where the stem cells of acute leukemia usually fail to divide. This growth difference in vitro is not an absolute one, since the Ph1 metaphases are found much more often in marrow aspirates than in cultured blood (1). In the blood cultures from eight patients we saw it with a frequency of only 20 per cent (68/317 cells); it was present in 81 per cent (244/302) of the metaphases from corresponding marrows (Sandberg et al., unpublished). For the above reasons, and despite its broad usefulness in medical genetics, the blood-culture method (16) cannot be relied upon in the karyotyping of acute leukemias and may give false negatives even for chronic myelocytic leukemia.

Future chromosome studies of acute leukemia will have to be based on examination of freshly fixed marrow aspirates. The advantages of the "direct" marrow method are self-evident:
1. It exposes the cytogenetic profile of the leukemic cell population in the patients' marrow and avoids false negative results which are the chief objection to reliance on blood cultures.
2. In vitro artifacts are ruled out and mitotic arrest by colchicine is not required.
3. Most marrow aspirates contain abundant mitoses, whereas cultures of leukemic tissue may grow poorly or not at all.
4. Chromosome findings in the marrow may provide new criteria for the classification of acute leukemias.
5. The karyotypic findings in the marrow permit meaningful correlation with clinical and histologic remission during therapy, and are of potential aid in prognosis.

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Fig. 1.—Hypo-diploid metaphase with 44 chromosomes from the marrow of 6-year-old male patient #1 with acute myeloblastic leukemia. Group G41-2 in this and most other modal cells of this marrow contained only two chromosomes instead of the normal four. X1200.

Fig. 2.—Presumably nonmalignant normal diploid metaphase with 46 chromosomes, which was the predominant cell type dividing in the blood culture of patient #1 and all other blood cultures from patients with acute leukemia. X1200.

Fig. 3.—Abnormal metaphase with 44 chromosomes, including a ring chromosome (arrow, see also Fig. 4), from the marrow of 62-year-old female patient #2 with untreated acute myeloblastic leukemia. X1200.

Fig. 4.—Hypo-tetraploid metaphase with 90 chromosomes—i.e., twice the modal number of 45 chromosomes which characterized the marrow of patient #2. Two prominent ring chromosomes are indicated by arrow. X1200.

Fig. 5.—Hypo-tetraploid metaphase with 85 chromosomes from the modal cell population of the marrow of 5-year-old female patient #3 with acute lymphoblastic leukemia. X2000.

Fig. 6.—Karyotype with 51 chromosomes, arranged from a modal metaphase predominating in the first marrow sample of 4-year-old male patient #4 with acute lymphoblastic leukemia. The five excess chromosomes at lower right include a large metacentric marker. X2000.
FIG. 7.—An apparently normal metaphase with 46 chromosomes in the second marrow sample of patient #4, who responded with clinical remission to leucrastine therapy. Note relatively sharp definition of chromatids. X1300.

FIG. 8.—A leukemic metaphase with the modal number of 51 chromosomes and the large metacentric marker (arrow) from the second marrow aspirate of patient #4. The somewhat fuzzy, swollen appearance of the chromosomes, by comparison with diploid metaphases in the same marrow, may indicate either an intrinsic chromatin difference between cells with 46 and 51 chromosomes, or a differential effect of leucrastine therapy. X1200.

FIG. 9.—Same as Figure 7.

FIG. 10.—Same as Figure 8.
FIG. 11.—A diploid, presumably nonleukemic, metaphase with 46 chromosomes at the left and a leukemic modal cell with 51 chromosomes including the large metacentric marker (arrow, or large unit at 7 o'clock) are here photographed side by side in the same microscopic field taken from the second marrow aspirate of patient #4. Note difference in the over-all appearance of the chromatids. X1200.

FIG. 12.—Pseudo-diploid modal metaphase with an abnormal set of 46 chromosomes from the marrow of 9-year-old male patient #6 with acute myeloblastic leukemia. The satellited acrocentric marker (arrow)—smaller than group D13-15 but larger than the normal Y—was present in 89 per cent of the marrow metaphases. X2000.
Chromosomal Dichotomy in Blood and Marrow of Acute Leukemia


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