Chromosome Studies on Normal and Leukemic Human Leukocytes [1]

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SUMMARY

Chromosome studies were made on myeloblasts obtained from the peripheral blood of 4 patients (3 males, 1 female) with acute or chronic granulocytic leukemia and on leukocytes from 3 healthy individuals (1 male, 2 females). The cells were grown in culture for 2 to 4 days before examination. Both in normal and in leukemic cells, the predominant metaphase chromosome number was 46. However, idiogram analyses revealed a definite abnormality, probably involving the Y chromosome, in the 2 cases of chronic granulocytic leukemia. No chromosome abnormality was distinguishable in the 2 cases of acute leukemia (1 male, 1 female) investigated. The results indicate that in some leukemic human leukocytes small but definite chromosomal changes are demonstrable.—J. Nat. Cancer Inst. 25: 85–109, 1960.

ALTHOUGH THERE have been numerous reports in recent years of chromosome abnormalities in a wide variety of malignant tumors, there is very little information on the chromosomes of leukemic cells in man. A few workers (*1*, *2*) have observed mitotic figures in direct smears or in tissue cultures of cells from the blood or bone marrow of patients with leukemia and have noted that in general the chromosome number appeared to be in the diploid range; earlier, Andres and Shiwago (*3*) described mitotic variations in leukemic cells similar to those seen in other malignant cells. However, until three very recent reports, no exact counts or analyses had been made.

Nowell *et al.* (*4*) obtained myeloblasts from the peripheral blood of 3 patients with acute granulocytic leukemia and, after short-term culture, found that the chromosome number agreed with the diploid value for man ($2n = 46$) and that no gross chromosomal abnormalities were present. More recently, Ford *et al.* (*5*) reported similar results from studies of bone marrow in short-term culture in one case of lymphocytic leukemia and

[1] Received for publication December 14, 1959.

[2] Supported in part by Senior Research Fellowship SF-4 from the Public Health Service and research grant C-3562 from the National Cancer Institute, National Institutes of Health, Public Health Service.

[3] Supported by a research grant from the American Cancer Society, Inc., to Dr. Jack Schultz.

[4] The authors wish to express their appreciation to Miss Juliet Goodfriend, Miss Sarah Reifsnyder, and Mrs. Elizabeth Krohnert for technical assistance.

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in another of blast-cell leukemia, but, in a third leukemia, of unspecified type, an abnormal number of 44 chromosomes plus 1 minute was observed. Baikie *et al.* (*6*) have just described chromosome studies in 11 cases of leukemia, employing the bone marrow technique. No aberrations were found in preliminary investigation of 6 cases of chronic leukemia, but in 5 cases of acute leukemia, there was abnormality of chromosome number in 1 case, abnormality of chromosome morphology in 2 cases, and abnormality of both number and morphology in the 1 case reported in detail.

In the present study, which is an extension of our previous report, the chromosomal characteristics of both normal and leukemic human leukocytes have been investigated by the use of short-term primary cell cultures, and it is believed, for reasons which will be discussed, that the results obtained accurately reflect the state *in vivo*. One of the 3 normal individuals described here (Normal case 1) was included in our earlier abstract, as were the 2 acute leukemia cases (1 and 2). In these cases, the preliminary data have been confirmed and amplified. Two additional normal cases and and 2 chronic leukemia cases are described here for the first time.

MATERIALS AND METHODS

Leukocytes were separated with phytohemagglutinin (Difco) from heparinized peripheral blood of healthy individuals and of patients with granulocytic leukemia. The cells were grown in short-term culture by a modification of Osgood's "gradient" method, which has been reported in detail elsewhere (*7*, *8*). Briefly, cells were grown in undisturbed deep cultures in a mixture of autogenous plasma and commercial medium (TC-199, Difco). Each culture bottle contained 1 or 2 slanted slides, and the cells settled out and grew on the slides as well as on the sides and bottom of the bottle. Mitoses appeared in the leukemia cultures on the 1st or 2d day, but were rarely observed in the cultures from normal individuals before the 3d day. In the leukemia cultures, nearly all of the inoculated cells were immature myeloid forms and were the source of the mitotic figures studied. The dividing cells in the normal cultures were derived from monocytes and perhaps large lymphocytes (*7*, *8*). Recent work (*9*) indicates that the phytohemagglutinin used in separating the leukocytes from whole blood is a specific initiator of mitotic activity among the normal leukocytes in these cultures.

Colchicine (1×10^{-7}M) was added at the time the cells were undergoing their first division in culture. Seventeen to 19 hours later, the cells were harvested from the bottom of the culture bottle, pretreated, fixed and stained in acetic orcein, and squash preparations were made (*7*, *10*). At the same time, the slides from the culture bottles were rinsed in distilled water, air-dried, and stained with Giemsa. The slides were used only for general survey purposes, while the actual chromosome counts and analyses were made on the squash preparations. In some instances, colchicine was

not used, so that anaphases and details of uncontracted metaphase chromosomes could be examined.

Idiograms were constructed from enlarged photomicrographs. In each case, the chromosomes have been classified into 3 groups, according to centromere position: median (top row, 7 pairs), subterminal (middle row, 6 pairs), and submedian (bottom row, 10 pairs). Within each of the 3 groups, the pairs have been arranged according to approximate order of decreasing length.

CASE HISTORIES

Chromosome studies were made on the leukocytes of 4 patients with acute or chronic granulocytic leukemia and 3 healthy individuals, members of the laboratory staff. Brief histories of the leukemia cases follow:

Leukemia case 1.—E. C., a 59-year-old white male, was admitted to Philadelphia General Hospital on July 25, 1957, because of weakness and fever of 1 month's duration. WBC = 37,000 (64% blasts); bone marrow diagnosis: "acute granulocytic leukemia." He was treated with 6-mercaptopurine and steroids during July and August, 1957, and from November, 1957, until his death, but never attained complete remission. White counts fluctuated from 3,000 to 130,000, with a high percentage of blast forms at all times. He died on April 17, 1958, after recurrent episodes of hemorrhage and infection. A total of 3 blood samples was obtained for chromosome study from this patient: 2 midway in the course of his disease (November 22, 1957, and December 2, 1957) and 1 during the terminal stages (March 28, 1958). White blood cell counts at those times were, respectively, 30,000 (92% blasts); 35,000 (88% blasts); and 52,000 (84% blasts).

Leukemia case 2.—I. K., a 35-year-old white female, was admitted to the Hospital of the University of Pennsylvania on October 12, 1957, with a history of weakness and purpura for 3 weeks. WBC = 12,000 (85% blasts and promyelocytes); bone marrow diagnosis: "acute granulocytic leukemia." Treatment with 6-mercaptopurine and steroids was started but was without effect. She died on October 16, 1957, with multiple hemorrhages. A blood sample for chromosome study was obtained on October 14, 1957; at that time WBC = 11,000 (91% blasts and promyelocytes).

Leukemia case 3.—E. K., a 41-year-old white male, whose case was first diagnosed as "chronic granulocytic leukemia" in April, 1955 (WBC = 318,000), was treated with Myleran and a remission was obtained which lasted until February, 1957. Exacerbation of the leukemia at that time was treated, with only partial success, by X-ray therapy to the long bones and with P^{32}. He remained reasonably well until July, 1958, when a second exacerbation of the disease failed to respond to further X-ray and Myleran therapy. He was admitted to the Hospital of the University of Pennsylvania August 13, 1958, and bone marrow examination confirmed the diagnosis of chronic granulocytic leukemia. He died of cerebral hemorrhage on August 27, 1958. A blood sample for chromosome study was obtained on August 19, 1958; at that time WBC = 375,000 (blasts and promyelocytes, 16%; myelocytes, 37%; metamyelocytes, 33%; segmenters, 12%; lymphocytes, 2%; monocytes, 0%).

Leukemia case 4.—G. H., a 33-year-old Negro male, was admitted to Philadelphia General Hospital on August 13, 1958, with a history of weakness and fatigue for 2 months. WBC = 248,000; bone marrow diagnosis: "chronic granulocytic leukemia." Myleran therapy was started on August 22, 1958. He was discharged, in remission, on September 17, 1958, and lost to follow-up. Blood for chromosome study was obtained on August 25, 1958; at that time WBC = 222,000 (blasts and promyelocytes, 17%; myelocytes, 16%; metamyelocytes, 14%; bands and segmenters, 31%; eosinophils, 7%; basophils, 10%; lymphocytes, 5%; monocytes, 0%).

RESULTS

Preliminary study of Giemsa-stained slides from both leukemia and normal cultures, neither of which had received colchicine, failed to reveal any gross mitotic abnormalities. Anaphases were entirely regular, without lagging or bridging of chromosomes. There were no spindle abnormalities such as multipolarity. Metaphase chromosome numbers were predominantly in the diploid range. Approximate counts of 200 metaphases on one slide (Leukemia case 1) gave a mean value of 44 chromosomes, with a standard error of ± 4. In both the normal and the leukemia cultures, approximately 2 percent of the metaphases were in the tetraploid range.

More precise studies on the squash preparations confirmed the impressions gained from examination of the Giemsa-stained culture slides. Chromosome counts were done on at least 30 metaphases in each case (tables 1 and 2); only those counts of metaphases which were accurate within 1 chromosome were accepted. A count recorded in the tables as 45 ± 1, 46 ± 1, 47 ± 1, or 48 ± 1 indicates that at one place in the metaphase plate it could not be definitely decided whether 1 or 2 chromosomes were present. The predominant chromosome number was 46 in all cases, both normal and leukemic.

TABLE 1.—Metaphase chromosome numbers of leukocytes from peripheral blood of normal humans

	Chromosome number					
Case number	45	45 ± 1	46	46 ± 1	47 ± 1	47
1	3	3	25	4	4	—
2	—	3	32	2	1	3
3	—	2	26	1	3	—

TABLE 2.—Metaphase chromosome numbers of leukocytes from peripheral blood of leukemic humans

	Chromosome number						
Case number	44	45 ± 1	46	46 ± 1	47 ± 1	47	48 ± 1
1	—	11	21	5	5	3	—
2	—	10	13	5	6	—	—
3	1	9	11	2	7	—	—
4	—	8	16	7	9	1	1

The tetraploidy which had been observed in both the normal and leukemia cultures was found, in the squash preparations, to be of two types, one showing endoreduplication (*11*) and one not; none of these was accurately countable and they are not shown in the tables. In addition, occasional apparently intact metaphases were observed in colchicine-treated material, *both* normal and leukemic, which had markedly *hypo-*

diploid chromosome numbers. Such a cell, with 28 chromosomes, is shown in figure 1. In one individual, not included in this report, idiogram analyses were made of 3 such metaphases, each having 10 chromosomes. There was little or no correspondence of chromosome types among the 3 and, in general, no pattern either of chromosome number or type was observed in other hypodiploid metaphases; their chromosome complements appeared to be derived at random from the normal complement. Since these hypodiploid forms were never observed in the absence of colchicine, it is thought that they represent a phenomenon induced by the relatively long colchicine treatments employed. Study of culture slides on which such cells appeared suggested that they resulted from a type of segmentation of cells arrested at metaphase. A stage in this process is shown in figure 2, a photomicrograph from a squash preparation; the 2 groups of chromosomes total 46.

These types of variation in chromosome number occurred with equal frequency in both normal and leukemic cultures. Of greater interest are the results of idiogram analysis, which revealed abnormalities of chromosome morphology apparently peculiar to cells in the 2 cases of chronic leukemia. Detailed descriptions of the results in each case follow.

Normal case 1.—White female, age 21. Metaphase chromosome numbers predominantly 46. Five idiogram analyses were made of cells with 46 chromosomes. Four of these were normal (figs. 3, 7), and 1 showed an abnormally long chromosome, apparently the result of a translocation. Of a total of 39 metaphases counted, 3 had 45 chromosomes. One of these was analyzed, and it was found that the missing chromosome belonged to the fifth or sixth longest pair of the group with submedian centromeres.

Normal case 2.—White female, age 48. Metaphase chromosome numbers predominantly 46. Three idiogram analyses were made of such cells and all were normal (figs. 4, 8). Of a total of 41 metaphases counted, 3 had 47 chromosomes. One of these was analyzed and found to have a large acentric or telocentric fragment in addition to a normal female complement (fig. 5).

Normal case 3.—Japanese male, age 31. Metaphase chromosome numbers exclusively 46, within the adopted limits of accuracy. A total of 31 metaphases was counted, of which 3 were analyzed (figs. 6, 9). No morphological irregularities were observed, and the Y chromosome (fig. 20a–c) compares well with descriptions of this chromosome by other workers (*5*, *13*, *14*).

Leukemia case 1.—White male, age 60. Metaphase chromosome numbers predominantly 46. Of 45 metaphases counted, 3 had 47 chromosomes. One of these 3 was analyzed, and an apparently normal complement was present, plus 1 minute chromosome. Five idiogram analyses were made of cells with 46 chromosomes and all were indistinguishable from normal (figs. 10,15, 21a–c).

Leukemia case 2.—White female, age 35. Metaphase chromosome numbers exclusively 46, within the error tolerated. Thirty-four met-

taphases were counted and 4 idiogram analyses were made; all were indistinguishable from normal (figs. 11, 16).

Leukemia case 3.—White male, age 41. Thirty metaphases were counted. Chromosome numbers were 46, within error tolerated, with the exception of a single count of 44 (idiogram analysis not possible). Analyses were made of 3 cells with 46 chromosomes. All idiograms revealed the absence of a normal Y and the presence, in its stead, of a chromosome less than half the size of the smallest autosomes (figs. 12, 17, 22a–c). This minute chromosome was recognized in many other metaphases that were not subjected to idiogram analysis.

Leukemia case 4.—Negro male, age 33. In 42 metaphases counted, chromosome numbers were predominantly 46, with 1 count of 48 ± 1 (idiogram not possible). Analyses were made of 5 cells with 46 chromosomes. One idiogram (figs. 14, 19) showed an apparently normal Y chromosome. The other 4 idiograms, however, showed, instead of the Y, a minute chromosome (figs. 13, 18, 23a–c) similar to that observed in Leukemia case 3.

Figures 20 to 23 provide a comparison of Normal case 3 (male) with Leukemia cases 1, 3, and 4 (males). The comparison is made to illustrate the relative sizes of the two smallest pairs of acrocentric chromosomes and the Y chromosome or the minute chromosome, whichever was present in each metaphase.

DISCUSSION

Although the metaphase chromosome numbers were predominantly 46 in all 4 cases of granulocytic leukemia investigated, definite abnormalities of chromosome morphology were present in 2 of the individuals. In the 2 cases of chronic leukemia in males, idiogram analyses revealed changes of a similar type involving the presence of a minute chromosome. These minutes, which were morphologically similar but not identical in the 2 cases, were apparently present in place of a normal Y, and are judged to have resulted from either a deletion of a portion of the Y chromosome or the replacement of the Y by an autosomal fragment. The possibility that the minute simply represents normal variation in the morphology of the Y chromosome seems unlikely since studies by other workers have not revealed such variation (*12*, *13*). Nor does it seem likely that the minute resulted from an abnormality of 1 of the 4 smallest acrocentric autosomes rather than of the Y, since none of the chromosomes interpreted in our material as being these 4 autosomes resembled a normal Y, such as was observed in our Normal case 3 and has been described by others (*12*, *13*). Interestingly, if the change observed does involve the Y chromosome, it is not correlated with sex linkage in the incidence of chronic granulocytic leukemia, since statistical studies show only slightly greater frequency in males than in females (*14*).

The chromosomes of the 2 cases of acute leukemia (1 male, 1 female) were indistinguishable from normal with respect to both number and

constitution. However, in the female, an alteration in chromosome morphology of the order of magnitude of that observed in the Y chromosome of the chronic leukemias might well be impossible to detect. In this connection it is, of course, possible that a variety of subtle cytogenetic changes [the "cryptostructural" rearrangements of Levan (*15*)] as well as purely genetic changes might be present in any of these leukemic cases and be undetectable by our methods.

On the basis of the few cases studied, no estimate can be made regarding the possible relationship of chromosome changes to the duration of the disease process or to the duration or type of therapy. However, in Leukemia case 1 no chromosome changes were present even after prolonged chemotherapy, while in Leukemia case 4 the minute chromosome was present although treatment had just been started. In the one case reported in detail by Baikie *et al.* (*6*), a female with acute granulocytic leukemia, the modal chromosome number changed after 7 months of steroid therapy, but abnormalities of both number and morphology were present before treatment was begun.

There seems little doubt that the "leukemic" chromosome patterns studied in the present paper were indeed derived from leukemic cells. Nearly all of the cells planted in the cultures from leukemic patients were immature myeloid forms; monocytes and large lymphocytes, the cells which divide in *normal* cultures, constituted no more than 5 percent of the original inoculum. Furthermore, the leukemic mitoses studied had usually occurred during the first 2 days in culture, whereas normal leukocytes were rarely observed in mitosis before the 3d day of culture. Finally, when permitted to mature *in vitro*, the dividing cells in the leukemia cultures developed into polymorphonuclear forms, while the dividing cells in the normal cultures differentiated into macrophages, multinucleated giant cells, and small lymphocytes (*8*).

The present study also provides data on the chromosomes of leukocytes of three normal individuals, one male and two female. Of particular interest in these cases is the low but definite incidence of chromosome abnormalities in these normal cells. Such abnormalities among other populations of normal human somatic cells have been reported previously by other authors. Counts other than euploid are regarded by Ford *et al.* (*5*) as artifacts of technique or interpretation. Tjio and Puck (*12*) state that of 2,000 cells, 99.9 percent had chromosome numbers of 46 or 92, with a tetraploid frequency of less than 3 percent. Chu and Giles (*13*) mention that in a few exceptional cells deviations of one chromosome from normal were observed. These were interpreted as representing either instances of somatic aneuploidy, originally present or induced during culture, or artifacts resulting from errors in technique. In the three normal cases presented here, there is no suspicion of pathological origin of the cells studied. The chance that such aneuploid numbers were induced during culture is remote, since only the first or, at the very most, the second mitosis *in vitro* is represented in these preparations. We believe that technical and interpretive errors have been

ruled out in the cases of aneuploidy and other chromosome aberrations in which idiogram analyses were made. While, as mentioned, it is not possible to estimate reliably in our preparations the frequency of such aberrations, it is reasonable to believe it is greater than 0.1 percent and probably not more than 2 percent.

The relationship of the present findings in human leukemia to the results of such investigations on other types of malignant tumors is not yet clear. Most previous studies of spontaneous mammalian cancers have revealed much greater variability of chromosome number and morphology than that reported here [*see*, for example Hsu (*16*), Koller (*17*)]. Leukemic cells are apparently not so far removed from their normal prototypes as the cells of many types of malignant tumors. For instance, leukemic "blasts" are essentially indistinguishable, both morphologically and biochemically (*18*), from the "blasts" of normal bone marrow, and *normal* as well as leukemic leukocytes have the capability, present only in *malignant* cells of other tissues, of detaching from their fellows and spreading throughout the body. Whether the more subtle chromosome changes observed in leukemic cells, as compared to other types of malignancies, are related to these biological differences has not yet been determined. Furthermore, the relationship of our results to those of Baikie *et al.* (*6*), who observed chromosomal abnormalities in acute leukemia, but not in chronic leukemia, cannot as yet be evaluated. The studies of chronic leukemia reported by Baikie *et al.* (*6*) were of a preliminary nature and might not have revealed the small chromosome abnormality observed in our chronic cases. Obviously, more extensive studies are needed on the chromosomes of various types of human leukemia and lymphoma, and such studies are planned. In addition, cytological investigations of other early primary neoplasms, both benign and malignant, such as the recent study by Palmer (*19*) of the Shope rabbit papilloma, may lead to a better understanding of the relationship of chromosome changes to the pathogenesis of tumors.

Notes added in proof:

1) A recent paper by Bayreuther (Nature 186: 6–9, 1960) indicates that the incidence of chromosome aberrations in a variety of early spontaneous and induced malignancies may not be as great as indicated by other workers.

2) A more detailed analysis of chromosomes in human leukemia will be provided by studies now in progress in our laboratories, employing an improved method of chromosome preparation from leukocyte cultures (Moorhead, P. S., Nowell, P. C., Mellman, W. J., Batipps, D. M., and Hungerford, D. A.: To be published).

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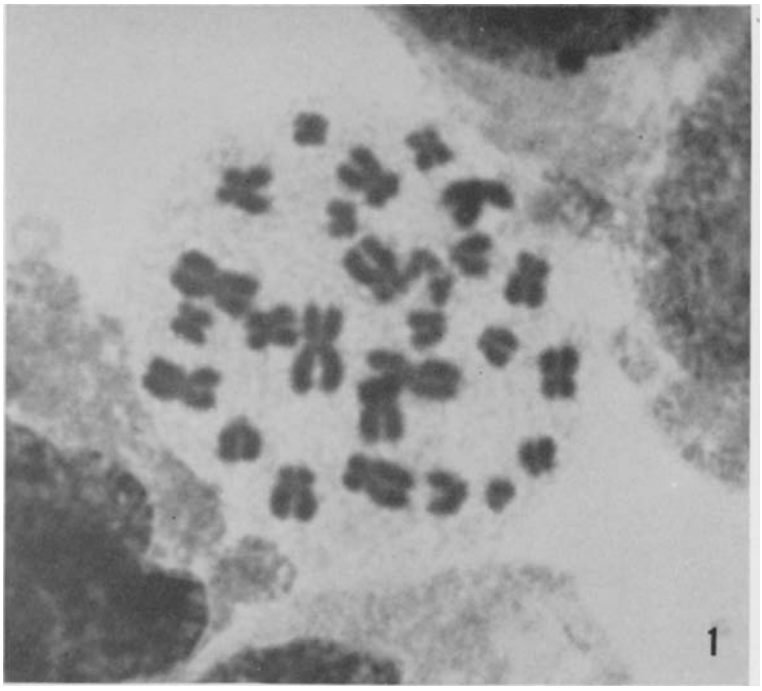
PLATE 11

FIGURE 1.—An apparently intact metaphase chromosome group with 28 chromosomes. Such metaphases were not observed in the absence of 17- to 19-hour colchicine treatment. × 2,700

FIGURE 2.—A stage in the formation of hypodiploid chromosome groups. The two groups of chromosomes total 46. × 2,700

PLATES



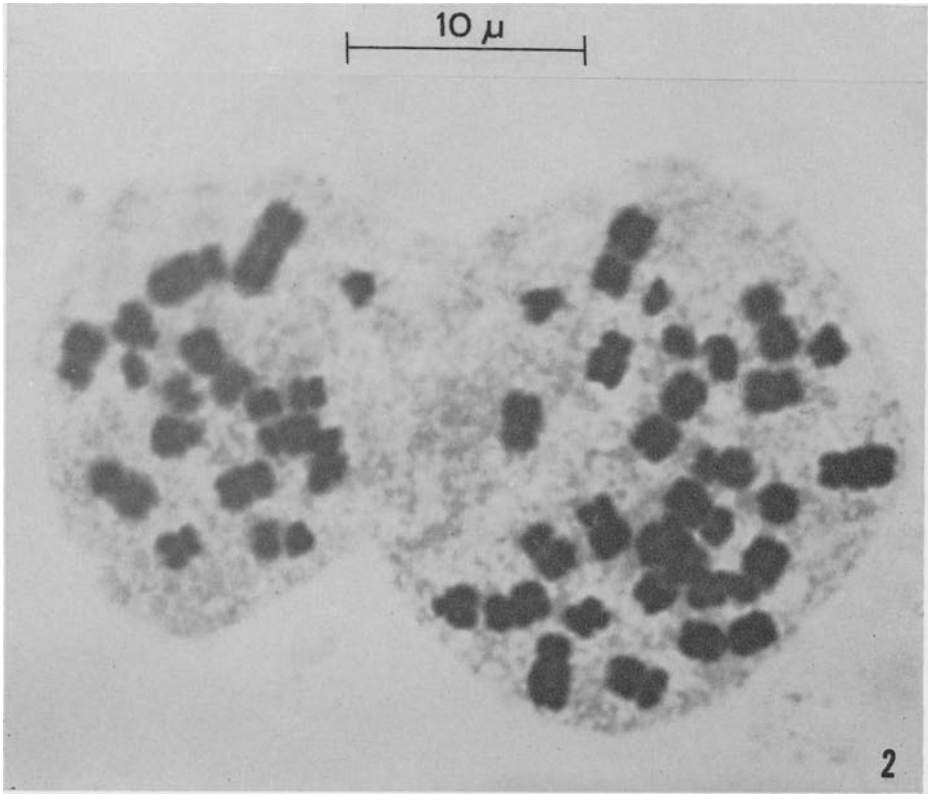


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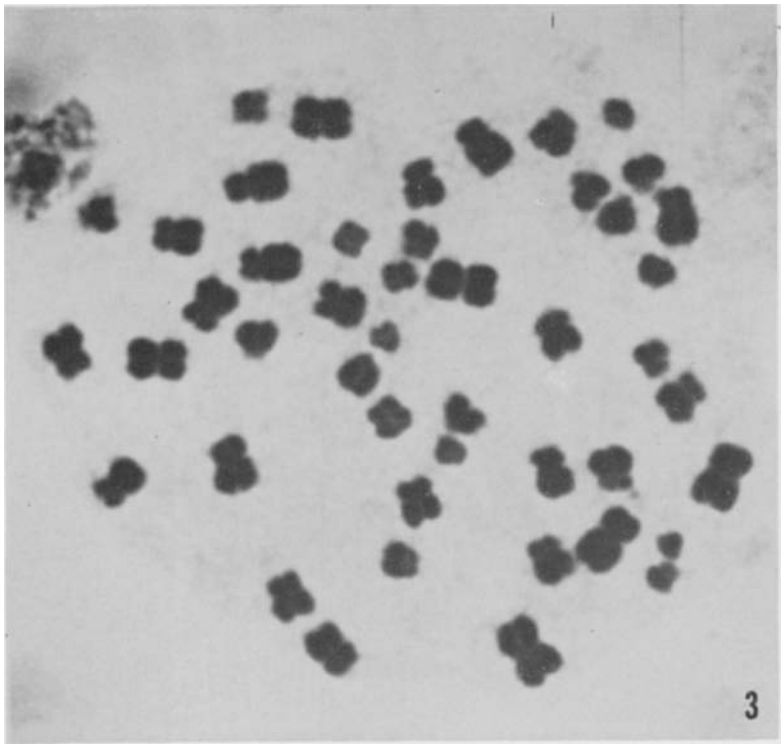
PLATE 12

FIGURE 3.—Metaphase from Normal case 1 (female), 46 chromosomes. *See* idiogram, figure 7. × 2,700

FIGURE 4.—Metaphase from Normal case 2 (female), 46 chromosomes. *See* idiogram, figure 8. × 2,700



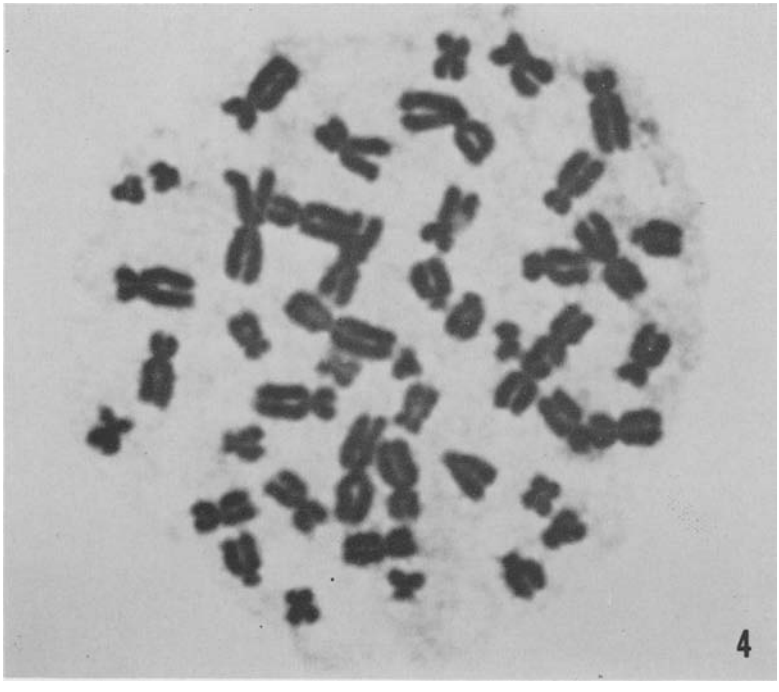
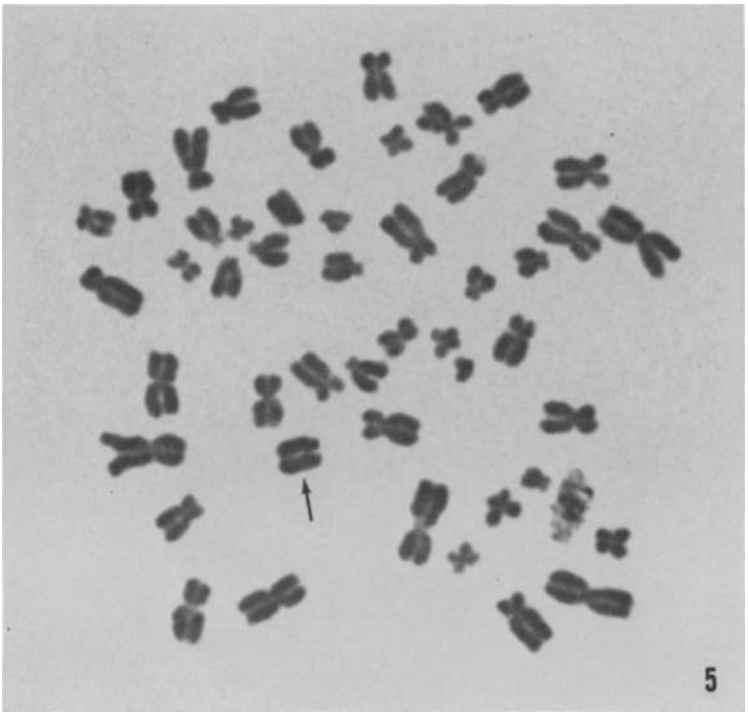


PLATE 13

FIGURE 5.—Metaphase from Normal case 2 (female), 47 chromosomes. *Note*, in addition to normal female complement, a large acentric or telocentric fragment (*arrow*). × 2,700

FIGURE 6.—Metaphase from Normal case 3 (male), 46 chromosomes. *Note* Y chromosome (*arrow*). *See* idiogram, figure 9. × 2,700



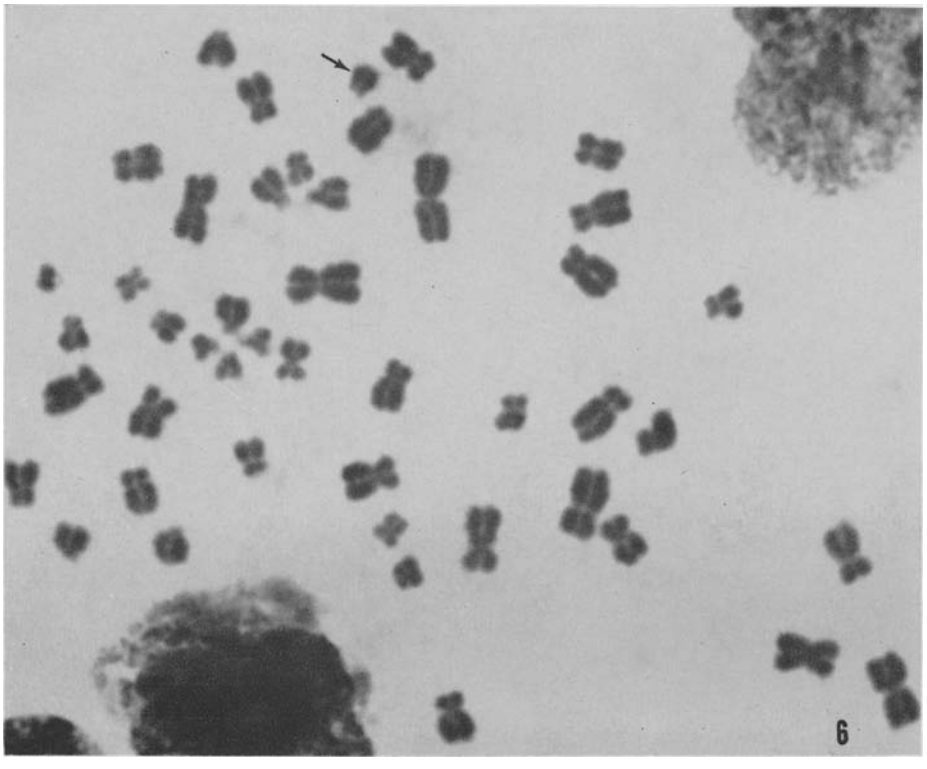


PLATE 14

FIGURE 7.—Idiogram of metaphase shown in figure 3. × 2,700

FIGURE 8.—Idiogram of metaphase shown in figure 4. × 2,700

FIGURE 9.—Idiogram of metaphase shown in figure 6. × 2,700

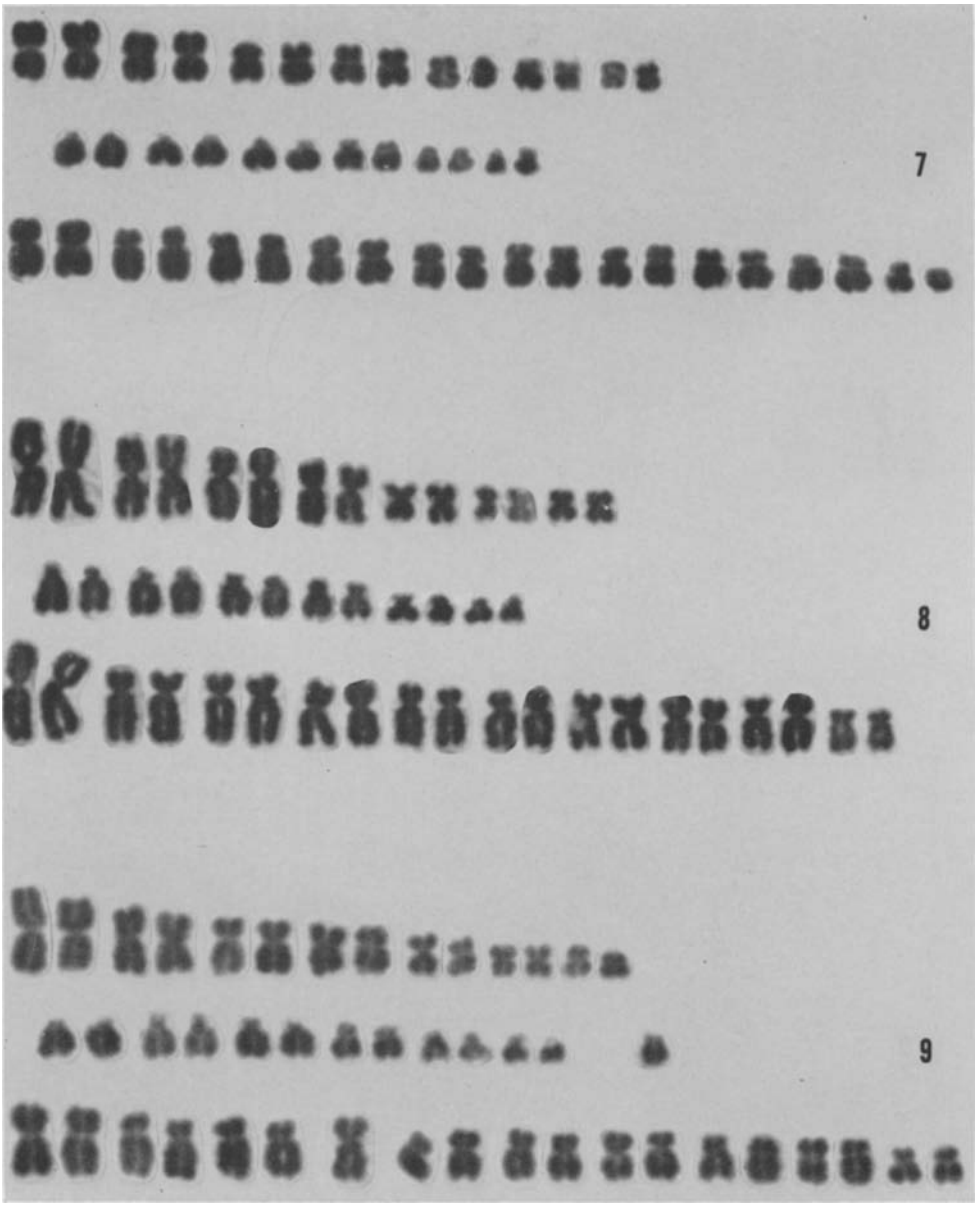
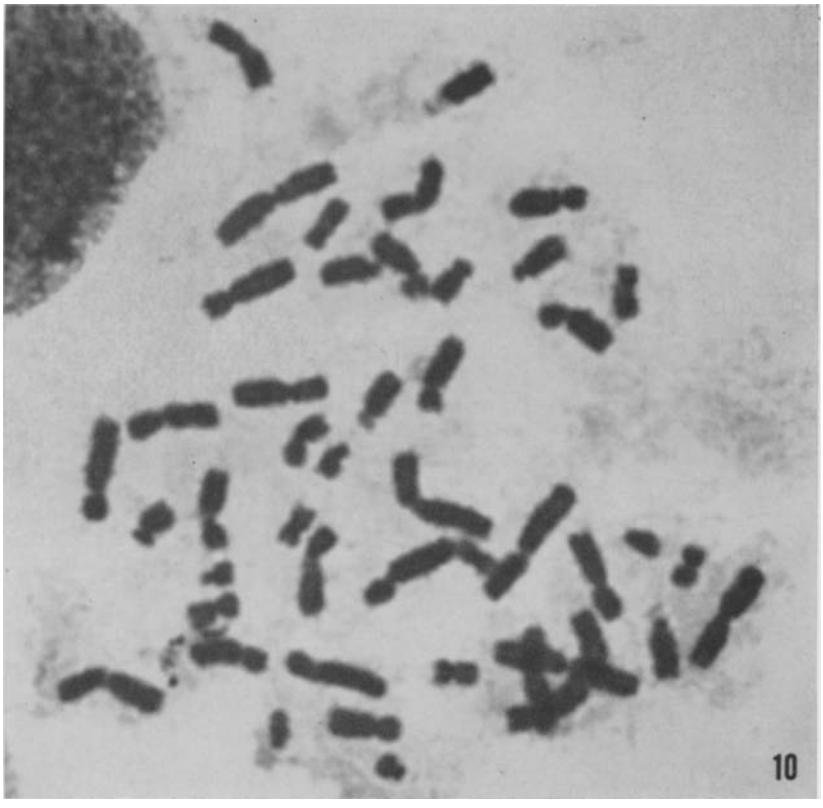


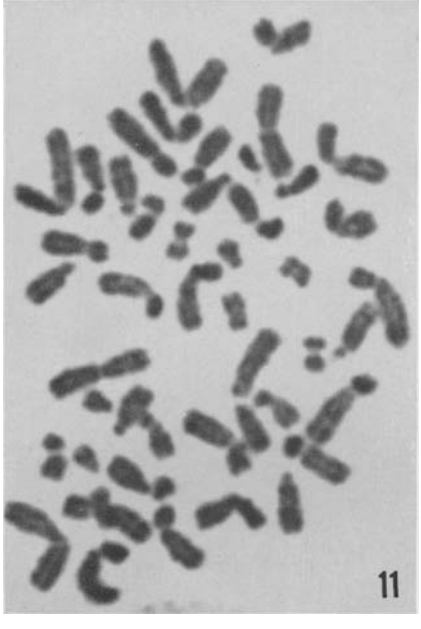
PLATE 15

FIGURE 10.—Metaphase from Leukemia case 1 (male), 46 chromosomes. *See* idiogram, figure 15. × 2,700

FIGURE 11.—Metaphase from Leukemia case 2 (female), 46 chromosomes. *See* idiogram, figure 16. × 2,700

FIGURE 12.—Metaphase from Leukemia case 3 (male), 46 chromosomes. *Note* minute chromosome (*arrow*). *See* idiogram, figure 17. All 3 metaphases analyzed from this case were similar. × 2,700





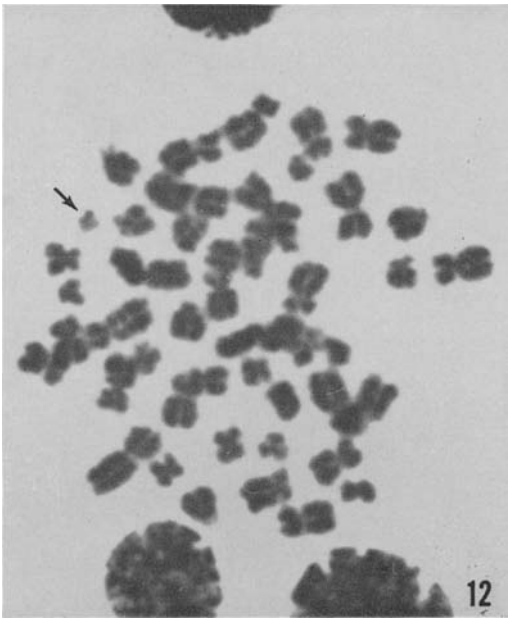
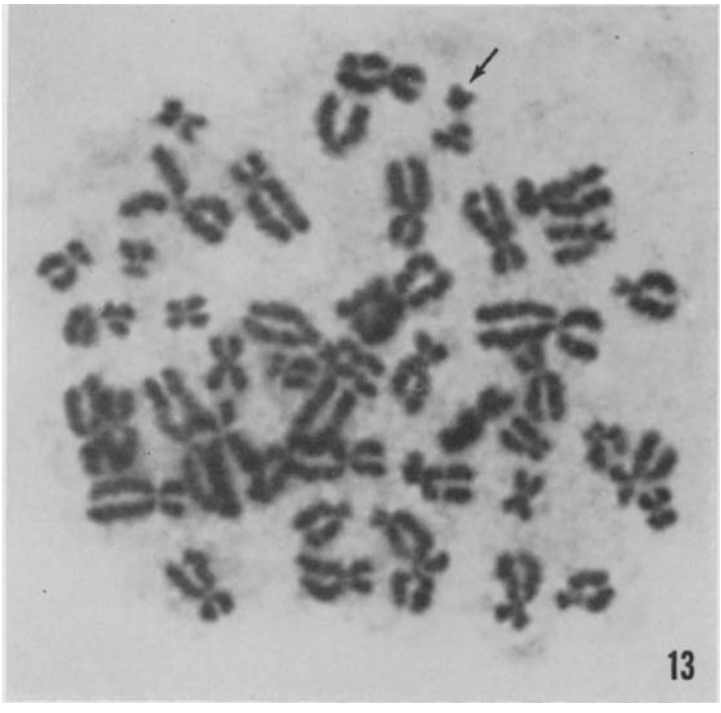


PLATE 16

FIGURE 13.—Metaphase from Leukemia case 4 (male), 46 chromosomes. *Note* minute chromosome (*arrow*). *See* idiogram, figure 18. × 2,700

FIGURE 14.—Another metaphase from Leukemia case 4, 46 chromosomes, no minute. *See* idiogram, figure 19. Of 5 metaphases that were analyzed, this is the only one with a normal male complement; all others were similar to figure 13. × 2,700



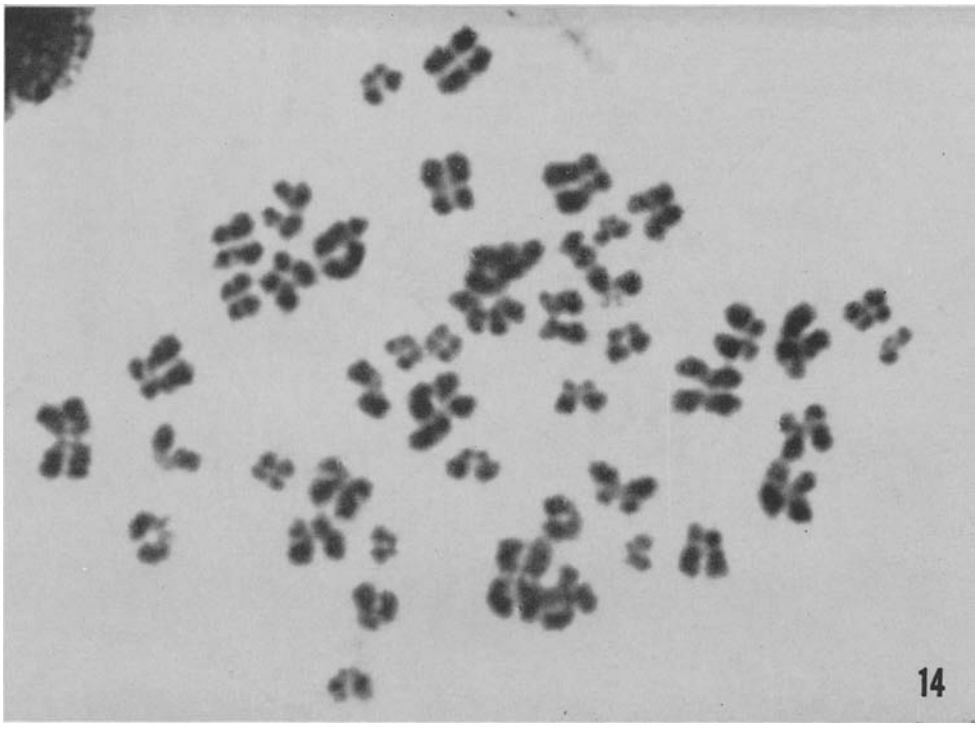


PLATE 17

FIGURE 15.—Idiogram of metaphase shown in figure 10. × 2,700

FIGURE 16.—Idiogram of metaphase shown in figure 11. × 2,700

FIGURE 17.—Idiogram of metaphase shown in figure 12. × 2,700

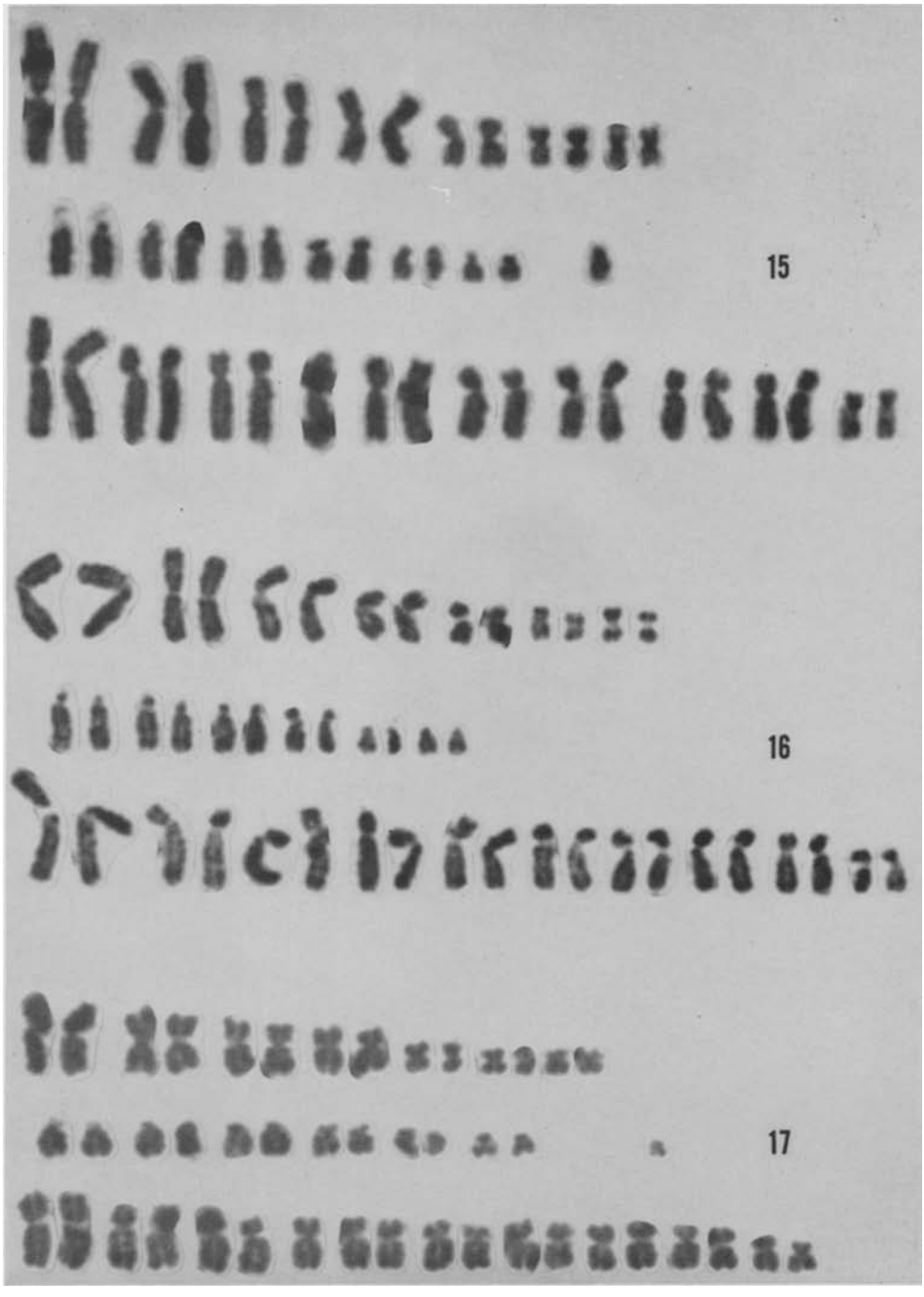


PLATE 18

FIGURE 18.—Idiogram of metaphase shown in figure 13. × 2,700

FIGURE 19.—Idiogram of metaphase shown in figure 14. × 2,700

A comparison of the four smallest acrocentric autosomes and the Y chromosome from a normal male (fig. 20a–c) with the same chromosomes from Leukemia case 1 (fig. 21a–c) and with the same autosomes and the minute chromosome from Leukemia case 3 (fig. 22a–c) and Leukemia case 4 (fig. 23a–c). × 2,700

FIGURE 20.—*a:* Chromosomes from figures 6 and 9. *b, c:* Chromosomes from two other metaphases of Normal case 3.

FIGURE 21.—*a:* Chromosomes from figures 10 and 15. *b, c:* Chromosomes from two other metaphases of Leukemia case 1.

FIGURE 22.—*a:* Chromosomes from figures 12 and 17. *b, c:* Chromosomes from two other metaphases of Leukemia case 3.

FIGURE 23.—*a:* Chromosomes from figures 13 and 18. *b, c:* Chromosomes from two other metaphases of Leukemia case 4.