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The Chromosome Constitution of Human Marrow in Various Developmental and Blood Disorders

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IMPROVED SPREADING TECHNIQUES for mammalian chromosomes have enlivened cytogenetic investigation of karyotype anomalies in developmental defects and nuclear variability in neoplasia. Recent controversy about the correct human somatic chromosome number (Tjio and Levan, 1956; Ford and Hamerton, 1956; Kodani, 1957; Stern, 1959a) now appears settled overwhelmingly in favor of 46 (Chu, 1960), although more meiotic data on Japanese individuals are needed to rule out somatic elimination of Kodani's supernumeraries (Makino and Sasaki, 1959). The diploid human idiogram has been elaborated in surprising detail; it permits identification of the X and Y chromosomes with a fair degree of certainty (Chu and Giles, 1959), and has served as the norm for the unravelling of apparent inconsistencies between the interphase sex-chromatin and phenotype (Barr, 1959 a and b).

The heredity of certain developmental anomalies which had defied decades of speculative debate was—entirely during the course of 1959—clarified in chromosomal terms, thanks to teamwork between physicians, geneticists and cytologists in a few English, French and Swedish laboratories. An undercurrent of international competition has accelerated the tempo of publications in this important enterprise. At any rate, England's "present lead in human chromosome studies" (Anonymous editorial, Lancet, 1959) seems a more civilized subject for national pride than championship in anti-human ballistics.

The priorities in the human chromosome field are recorded in an excellent recent discussion by Nachtsheim (1959). There is, however, need for a more inclusive summary, such as the present compilation of our findings in 42 human marrows with all available data on various developmental disorders and leukemias.

METHODS

Marrow cells were secured by aspiration of the sternal or iliac marrow. The puncture area was infiltrated with novocaine and 1 to 2 ml. of marrow was

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drawn into a sterile syringe. In the study of developmental anomalies (but not for the leukemic preparations) we employed the short-term culture method of Ford, Jacobs and Lajtha (1958), with the following modifications: Earle's solution was used instead of Krebs-Ringer, and the patient's own serum was added to the 5% glucose in isotonic saline, in place of AB serum. Marrow specimens were obtained during the afternoon; they were then held for about 9 hours at room-temperature in a waterbath in which a heating and recirculation device turned itself on at 1:30 a.m., quickly achieving a constant temperature of 37°C ±0.5°C. After 7 hours, sufficient colchicine was added to make a culture concentration of 0.005%, and incubation was continued for a further 1½ to 2 hours. No growth failures occurred in any of the marrows so processed. Incubation was followed by brief treatment with hypotonic citrate. Fifty per cent acetic acid gave better fixation than acetic alcohol. Acetic orcein was used for staining in preference to the more cumbersome Feulgen technique. Orcein staining was also found more suitable than the Schiff reaction for demonstration of X-chromosomal heteropycnosis.

After failing with the above method to obtain sufficient numbers of leukemic mitoses for chromosome study, the leukemic marrows were not incubated, but were aspirated into Earle's solution, immediately treated with hypotonic citrate, fixed and squashed without prior use of colchicine. Well-spread, countable metaphases, representative of the marrow population components existing *in vivo*, were usually obtainable by this direct technique.

Sex-chromatin and "drum-sticks" were examined in smears of buccal mucosa and blood, whenever necessary for diagnostic purposes.

RESULTS AND DISCUSSION

1. Normal marrows

Marrow cells from 10 normal subjects, 5 men and 5 women, had the diploid chromosome number of 46 in 90% of 307 exact metaphase counts. The chromosome counts in the diploid range appear on line 1 of Table 1. Figures 1 and 2 depict the normal female and male karyotypes.

All marrows contained up to 4% polyploid metaphases, ranging from tetraploid to 32-ploid. The polyploid cells were interpreted as dividing megakaryocytes or osteoclasts (Fig. 3). Somatic pairing of homologous chromosomes in diploid cells occurred with a frequency of about 0.3%. In these metaphases, the haploid number of bivalent chromosome pairs was established.

Figure 4 is from a normal female marrow and shows altogether 23 bivalents. It should be noted that the thickness of these laterally paired units is considerably greater than in diploid metaphases, such as Figures 9–14, which are reproduced at the same magnification. Twenty-four units can be distinguished in a similar cell (Fig. 5) from the marrow of a Klinefelter's patient (see below) with a chromosome constitution of 47 (XXY). Judged by their thickness, there are 21 autosomal bivalents. The 2 smallest autosomal homologues, situated near upper center and at about 7 o'clock, have failed to pair. The probable XXY

TABLE 1. BONE MARROW CHROMOSOME CONSTITUTIONS IN NORMAL SUBJECTS AND VARIOUS DEVELOPMENTAL DISORDERS

(based on 1458 exact counts in the diploid range)

Number of cases	Diagnosis	Total metaphase counts	% Frequency of chromosome numbers 43	44	45	46	47	48	49
10(5♀, 5♂)	Normal controls	307	—	3	5	90	2	—	—
7*	Turner's (XO)	444	0.5	5	86	8	0.5	—	—
1*	Turner's (XO and XX?)	34	6	2	50	42	—	—	—
1*	Turner's (XO or XX?)	110	—	1	49	45	4	1	—
1*	Turner's (XO)	30	—	—	10	70	13	7	—
4†	Klinefelter's (XXY)	86	—	—	—	1	87	11	1
2*	Testicular deficiency (XY, XXY?)	100	—	1	4	57	38	—	—
1*	Intersex (XY)	85	—	5	13	82	—	—	—
1♀†	Precocious puberty	54	—	—	2	22	52	22	2
1♀†	Precocious puberty	27	—	—	8	88	4	—	—
2♂*	Mongolism	86	—	—	1	8	90	1	—
2♂*	Lowe's syndrome	29	—	—	3	97	—	—	—
1♀†	Pseudohypoparathyroidism	66	—	2	6	90	2	—	—

* = buccal mucosa negative for sex-chromatin
† = buccal mucosa positive for sex-chromatin

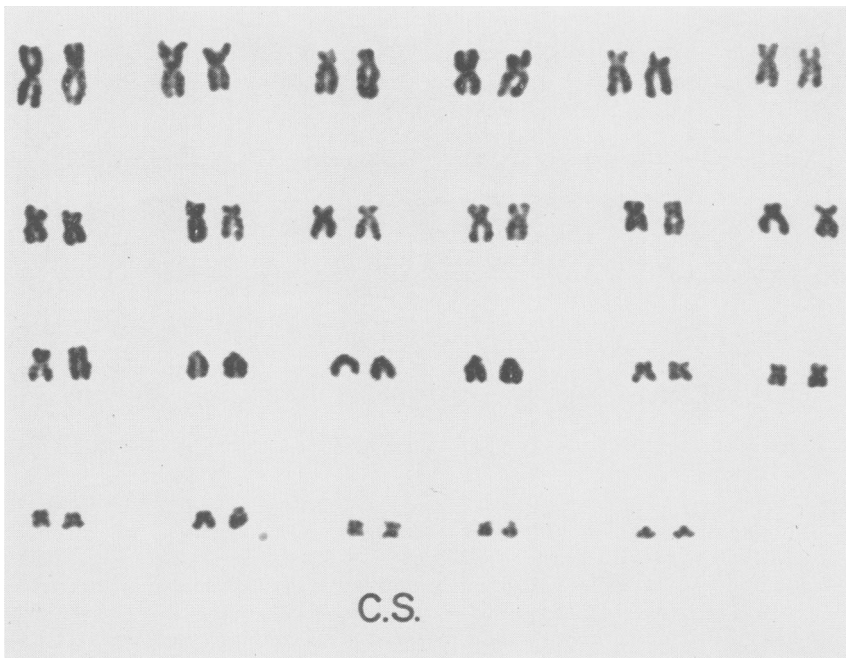

FIG. 1—Diploid idiogram (2n = 46) from marrow of C.S., a normal female. The first chromosome pair in the second row is probably the X pair. (× 2100).

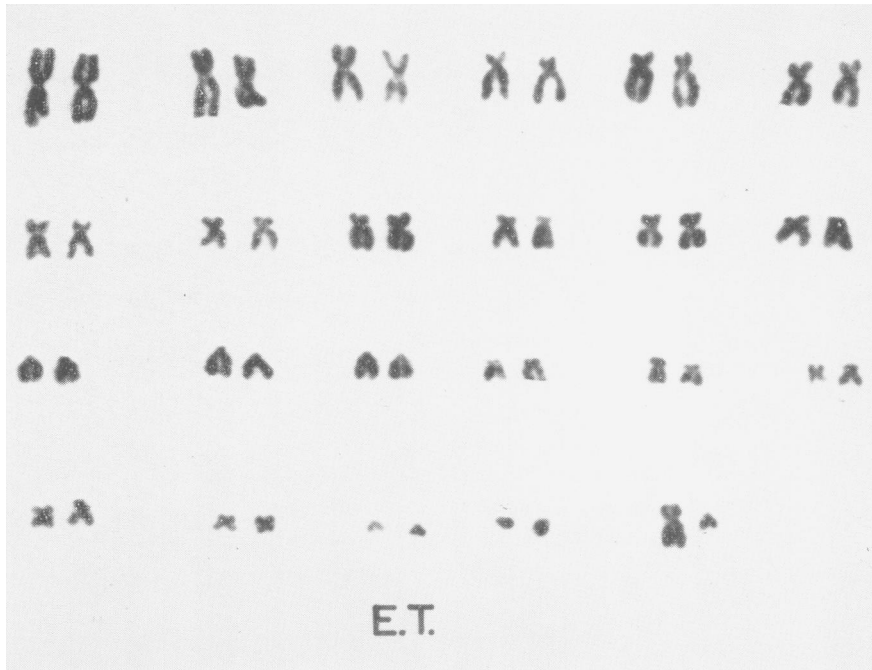

FIG. 2—Diploid idiogram (2n = 46) from marrow of E.T., a normal male. The fifth chromosome pair in the bottom row is interpreted as XY. (× 2200).

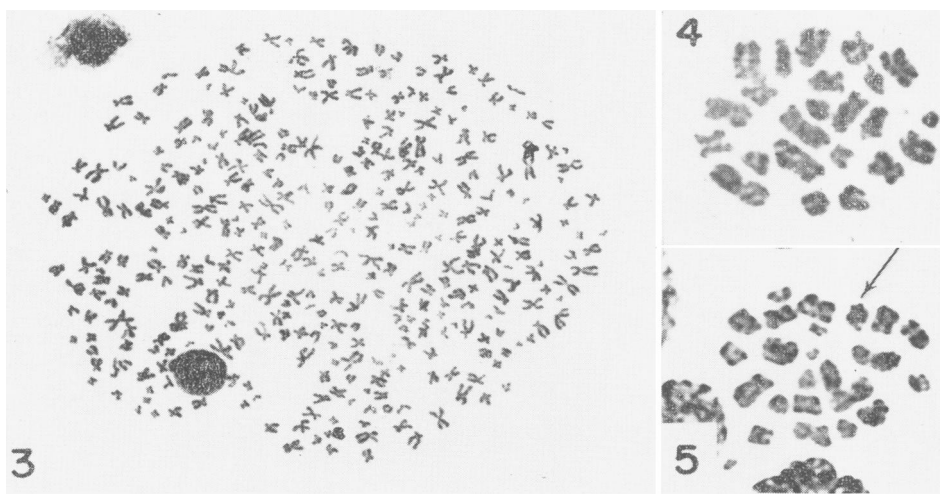

FIG. 3—Polyploid metaphase (16n − 8 = 360) of megakaryocyte or osteoclast from marrow of J.M., a patient with Turner's syndrome. (× 300).

FIG. 4—Somatic pairing of homologous chromosomes in a diploid marrow cell of C.S., a normal female. The haploid number of 23 bivalents may be counted. Note that thickness of paired chromosomes is much greater than in the diploid metaphases Figs. 9–14 which are reproduced at about the same magnification. (× 1200).

FIG. 5—Somatic pairing of homologues in a diploid marrow cell of N.W., a patient with Klinefelter's syndrome. This metaphase plate is interpreted as being composed of 24 units: 21 bivalent autosome pairs, 2 small unpaired autosomes and the XXY trio (arrow). (× 1200).

trio—2 laterally paired X-chromosomes and the small Y, terminally attached to the left-hand X as in male meiosis—is marked by an arrow. Figures such as these are rare and their interpretation is, of course, debatable. However, they have been observed and analyzed in detail often enough to rule out the possibility that they are accidental approximations of the haploid number resulting from asymmetric mitosis or broken metaphase plates.

Similar "haploid" mitoses were described in rat myelocytes by Kinosita et al. (1954). Since no haploid prophases were found in our human marrows, somatic pairing in diploid nuclei is either an abortive phenomenon, or it may initiate somatic reduction to haploidy followed by nuclear degeneraton, such as occurs in normoblastic maturation.

2. Marrow karyotypes in abnormal developmental conditions

A. Ovarian Agenesis (Turner's Syndrome): Our criteria for diagnosing Turner's syndrome were threefold: no evidence of ovarian function, buccal mucosa negative for sex-chromatin, certain accessory anomalies (webbing of the neck, cubitus valgus, congenital heart disease, growth retardation). Since the latter conditions vary greatly from case to case, somatic mosaicism for the XX and XO condition was considered as a possible contributory factor to this variability. However, the most obvious explanation for the diverse clinical manifestations is phenotypic expression of a variety of recessive traits carried on the single X, 45 (XO).

Difficulties in the precise identification of the submetacentric human X-chromosome, which ranks about 5th to 7th in length, have been admitted by most cytologists. In Turner's and in Klinefelter's syndrome, and less distinctly in normal female tissues, this difficulty is to some extent overcome by the pronounced heteropycnosis and allocycly of the X, not previously seen in man. An allocyclic replication and contraction rate is to be inferred from the characteristic staining properties of the X during different mitotic stages in certain female tissues of mouse, rat, Chinese hamster and other mammals (Ohno et al., 1959; Ohno and Hauschka, 1960; Kato and Yerganian, 1959). For a more detailed discussion of this allocycly, recently demonstrated by differential uptake of tritium thymidine in the X-chromosomal DNA of the grasshopper (Lima de Faria, 1959) and the hamster (Taylor, quoted by Yerganian, 1959), the paper by Ohno and Hauschka (1960) should be consulted.

Two extremes of X-allocycly may be seen in the idiograms of Figures 6 and 8. Figure 6 shows the single heteropycnotic, tightly contracted and intensely stained X on the right of the third row. Figure 8 shows abnormal lagging in the replication of the last chromosome in row 6, identified as the X. The doubling of all the autosomes in this metaphase is clearly complete, while the X appears to have failed in replicating its DNA. Usually, X-allocycly is less extreme, as apparent from Figures 9–13. In late prophases and beginning metaphases, the most intensely stained unit often meets the criteria for arm-length and size described for the X by Chu and Giles (1959). Heteropycnosis, therefore, may serve as an additional useful (though not unequivocal) attribute in the identification of the sex-chromosomes.

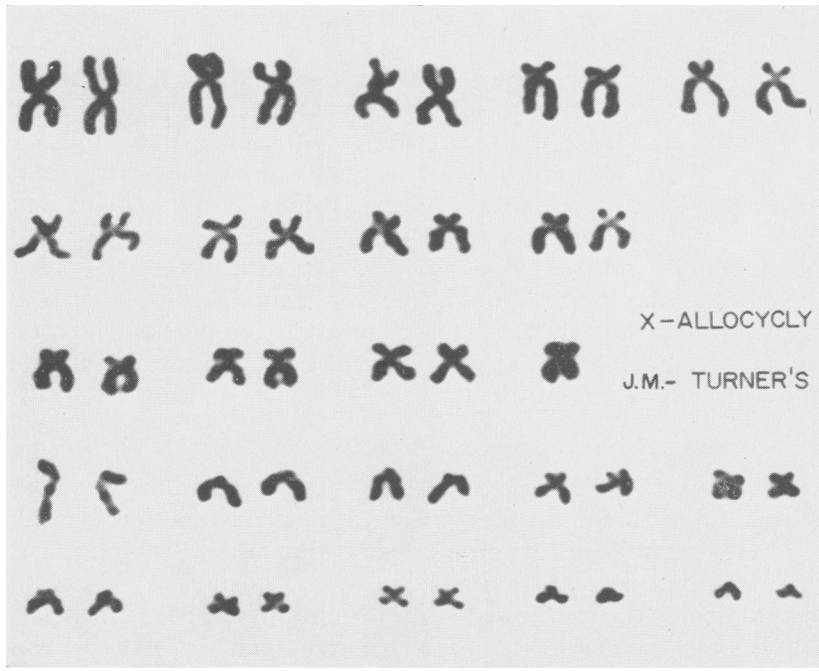

FIG. 6—Hypodiploid idiogram ($2n - 1 = 45$) from marrow of J.M., a patient with Turner's syndrome. The single, heteropycnotic X-chromosome lies at the right of the third row. This idiogram was prepared from the metaphase shown in Fig. 10. (× 2000).

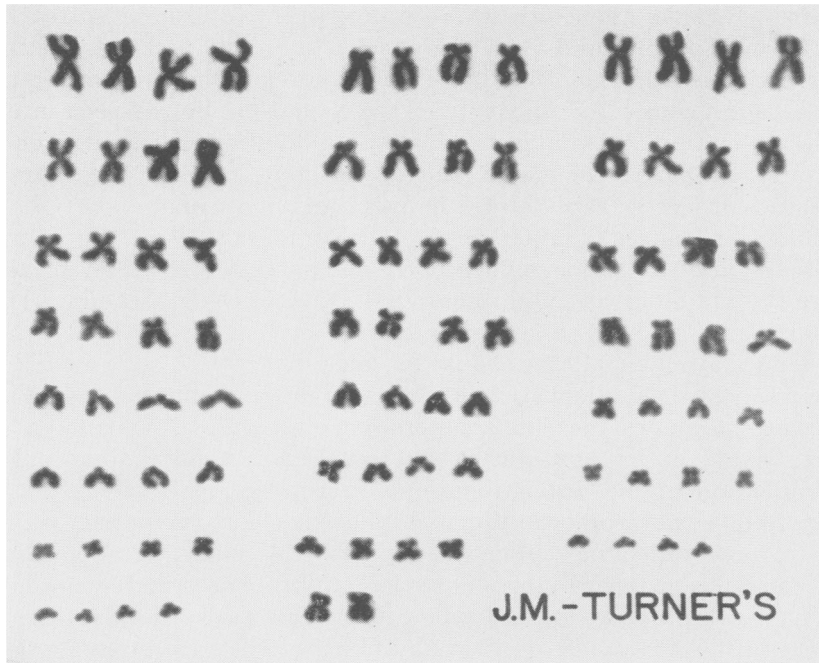

FIG. 7—Hypotetraploid idiogram ($4n - 2 = 90$) from marrow of J.M., a patient with Turner's syndrome. The two chromosomes at the right of the bottom row are believed to be the two X-chromosomes. (× 1400).

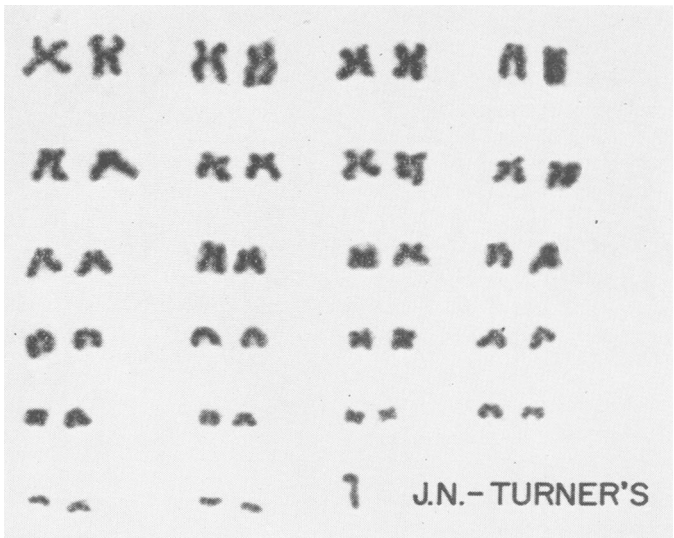

FIG. 8—Hypodiploid idiogram ($2n - 1 = 45$) from marrow of J.N., a patient with Turner's syndrome. Extreme allocycly in the replication rate of the single X-chromosome is evident from the as yet not clearly divided X at the right of the bottom row. As a rule, the X does not lag nearly as far behind the autosomes. (× 1600).

We have analyzed a representative series of 10 Turner's patients (Table 1, lines 2–5). In 7 of them, the modal chromosome constitution was 45 (XO), as first described by Ford et al. (1959b) and subsequently by others (Table 2, lines 1–5, Figs. 6, 7, 8).

In 2 of our subjects with the characteristic syndrome of ovarian agenesis, the chromosome constitution was about half 45 (XO) and half 46 (presumably XX). The 2 major karytoypes in the mosaic marrow on line 4 of Table 1 were imbalanced not merely with regard to the X-chromosome (either XO, or normal X plus incomplete X^1, as indicated by arrows in Fig. 11); they were either monosomic or trisomic for one of the smallest autosomes, as evident from the uneven number of small chromosomes in 10 metaphases suitable for detailed analysis.

On clinical grounds, the 38-year-old individual listed on line 5 of Table 1 was an unusual Turner's patient: 6 feet tall, muscular, weighing 214 pounds. While only 10% of her marrow cells contained 45 chromosomes, she was definitely of the XO constitution, the modal number of 46 resulting not from XX, but from trisomic representation of one of the two *largest* autosomes. This case will be studied further to determine whether her exceptional marrow idiogram extends to other somatic tissues.

During the "Second Symposium on Nuclear Sex" at King's College, London, Ford's discussion of 1 certain and 2 possible XX/XO mosaics with Turner's syndrome stimulated an "acute difference of opinion" (Anonymous editorial, Lancet, 1959). This skepticism concerning somatic mosaicism in man is no longer justified. Besides Ford's 3 cases, there are now on record 4 mosaic marrows

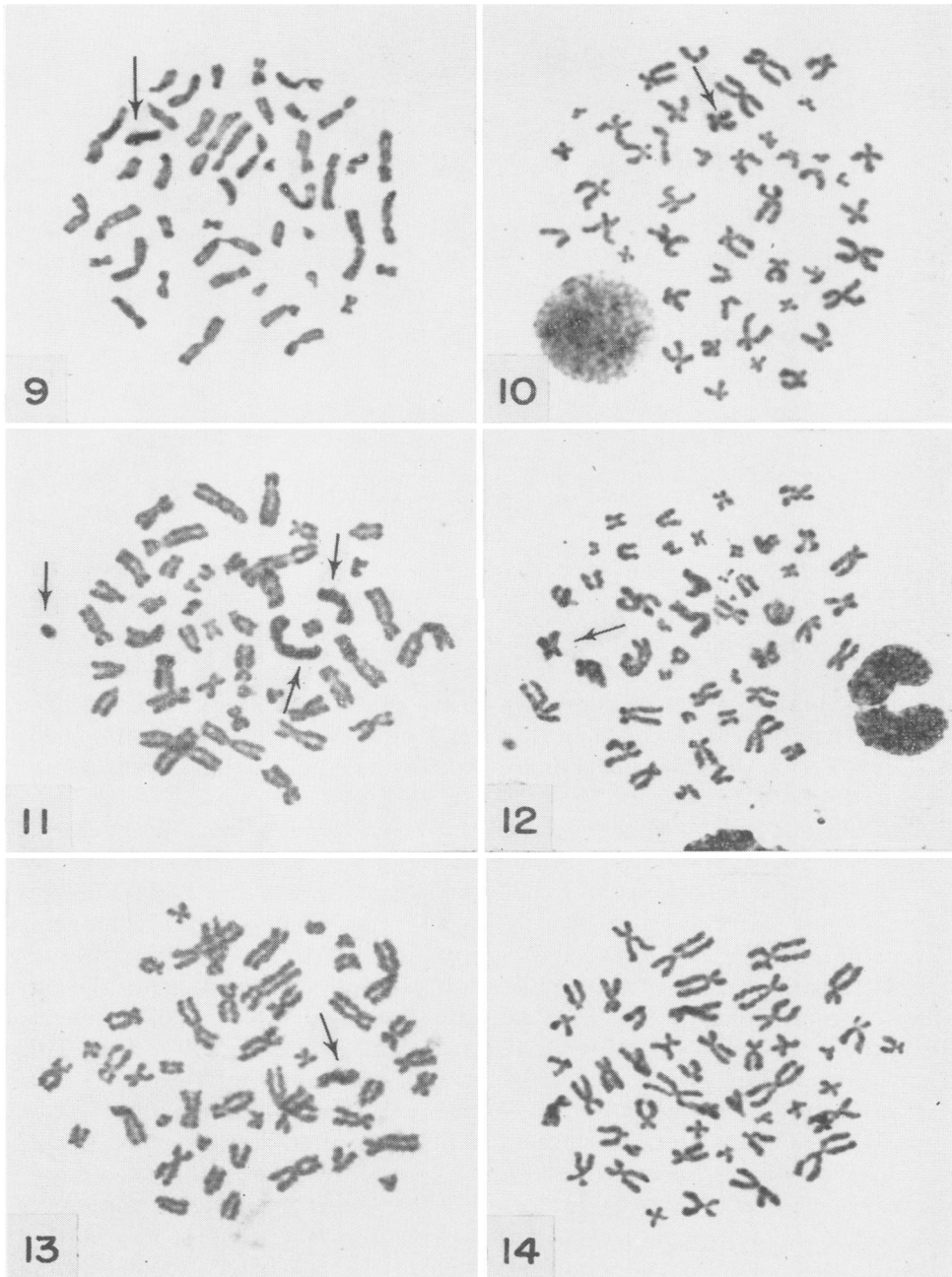

FIG. 9—Hypodiploid pro-metaphase (2n − 1 = 45) from marrow of J.M., a patient with Turner's syndrome. Arrow points to the heteropycnotic X-chromosome. (× 1500).

FIG. 10—Hypodiploid metaphase (2n − 1 = 45) from marrow of J.M., a patient with Turner's syndrome. Arrow points to the heteropycnotic X-chromosome. (× 1500).

TABLE 2. SUMMARY OF CHROMOSOME CONSTITUTION IN 19 CASES OF TURNER'S SYNDROME

Number of cases	Sex chromatin		Cytologic findings		Reference
	Buccal mucosa or skin	Blood	Characteristic chromosome number	Sex chromosomes	
1	—	n.t.*	45(98%)	XO	Ford et al., 1959b
1	—	n.t.	45(96%)	XO	Jacobs and Keay, 1959
2	—	n.t.	45	XO	Fraccaro et al., 1959
1	—	n.t.	45(100%)	XO	Tjio et al., 1959
3	—	n.t.	45	XO	Fraccaro et al., in press
1	—	n.t.	45 and 46	XO, XX(?)	" " " in press
7	—	+ or −	45(86%)	XO	Present report
2	—	+	45(50%), 46(43%)	XO and XX(?)	" "
1	—	++	45(10%), 46–48 (90%)	XO	" "

* = not tested

from patients with ovarian agenesis (Table 2, lines 6, 8, 9) and 3 mosaics in Klinefelter's syndrome (Table 3, lines 4, 7). Thus, a fairly high proportion of these abnormal individuals exhibits karyotypes that presuppose *somatic* nondisjunction during embryogenesis or during later life, possibly superimposed on sex-chromosomal abnormalities originating from *meiotic* nondisjunction.

While sex-linked marker genes may tell us which X-chromosome has been eliminated, they allow no absolute decision between elimination in a parental gonad or during ontogeny. Since the observed somatic mosaicism may well extend into tissues other than the marrow, it detracts somewhat from the reliability of sex-linked recessive color-blindness as evidence for X-chromosomal nondisjunction during maternal vs. paternal *meiosis*. That both may occur seems, nevertheless, clear from the hereditary analyses of Stern (1959b) and Stewart (1959).

Our own series includes a colorblind Turner's individual whose brother and maternal grandfather had defective red-green color vision, but whose mother

FIG. 11—Hyperdiploid metaphase (2n − 1 + 2 = 47) from mosaic marrow of G.G., a patient with Turner's syndrome. The arrow at the left points to a small chromosome for which the cells of this patient are either tri- or mono-somic. Arrow at upper right indicates the heteropycnotic X-chromosome, a piece of which may have been translocated to the larger heteropycnotic unit below it, which is perhaps a second X. Another possible interpretation is that this metaphase is XO, in which case the stem-cell type with 47 chromosomes would be trisomic for one of the four largest chromosomes. (× 1500).

FIG. 12—Diploid metaphase (2n = 46) from marrow of N.W., a case with testicular deficiency. The shape and heteropycnosis of the unit indicated by arrow suggests that it is the X-chromosome. (× 1500).

FIG. 13—Diploid metaphase (2n = 46) from marrow of N.W., a case with testicular deficiency. The heteropycnotic X-chromosome is indicated by arrow. (× 1500).

FIG. 14—Hyperdiploid metaphase (2n + 1 = 47) from marrow of D.L.S., a patient with Klinefelter's syndrome. (× 1500).

TABLE 3. SUMMARY OF CHROMOSOME CONSTITUTION IN 15 CASES OF KLINEFELTER'S SYNDROME, TESTICULAR DEFICIENCY, AND INTERSEX

Number of cases	Sex chromatin in buccal mucosa	Cytologic findings		Reference
		Characteristic chromosome number	Sex chromosomes	
1	+	47	XXY	Jacob's and Strong, 1959
4	−	46	XY	Jacobs et al., 1959c
1 (also a Mongol)	+	48	XXY	Ford et al., 1959a
1	+	46 and 47 (68%)	XXY and XX(?)	Ford et al., 1959c
1	−	46	XY	Ford et al., 1959c
4	+	47	XXY	Present report
2	−	46(57%) and 47	XY and XXY(?)	" "
1 (Intersex)	−	46	XY	" "

(a carrier) and father had normal vision. This patient's marrow contained more than 90% 45 (XO) cells. The single X-chromosome was obviously inherited from her mother. In this instance it may be claimed that fertilization of a normal ovum by an O-sperm, produced after nondisjunction or asynapsis of XY during paternal meiosis, was responsible for the XO constitution.

B. Testicular Dysgenesis (Klinefelter's Syndrome) and Testicular Deficiency: Only 4 patients with definite physiological and anatomical deficiencies of the testes, and at the same time showing positive sex-chromatin in their buccal mucosa, are here classified as Klinefelter's syndrome (Table 1, line 6). In the marrows of these subjects, the expected mode of 47 (XXY), as first established by Jacobs and Strong (1959), predominated (Fig. 14). A testicular biopsy from one of these individuals contained no trace of spermatozoa, spermatogonial mitoses or meiotic stages.

While 5 cases of "testicular feminization", examined by Jacobs et al. (1959c) and Ford et al. (1959c) were consistently 46 (XY) with negative buccal mucosa, our 2 subjects with testicular deficiency (not feminized) had mosaic marrows (Table 1, line 7). The modal cell type, shown in Figures 13 and 14, was 46 (XY); but more than 30% of the metaphases had 47 chromosomes and probably contained XXY. These two interesting cases resemble Klinefelter's syndrome clinically and suggest the possibility of somatic loss of one of the two X's.

Table 3 summarizes the pertinent data for 15 cases in this general category.

C. Precocious Puberty and Intersex: In one case of precocious puberty (Table 1, line 10), marrow had the normal chromosome constitution of 46 (XX). Another such patient examined by us had a record of menses and breast development since birth (Table 1, line 9). About half of her marrow metaphases had 47 chromosomes, the rest were evenly divided between 46 and 48. The modal cell type was trisomic for a chromosome in the general size-class of the X, and the patient may be tentatively considered as a triple-X female. By physiological and anatomical standards, this 9-year-old girl is better qualified for the designation "super female" than the masculinized patient shown by Jacobs et al. (1959b).

The marrow of an 18-year-old subject with intersex and negative buccal mucosa had a chromosome complement of 46 (XY). At laparotomy the following organs were identified: bilateral testes, bilateral fallopian tubes without a uterus, definite ovarian tissue on the right side and questionable ovarian tissue on the left. The external genitalia had intersexual aspects and there was no breast development. The XY karyotype of this individual, shown in Figure 15, differs from the only other intersex in the literature (Hungerford et al., 1959) where the chromosome constitution was undoubtedly 46 (XX).

D. Mongolism and Other Anomalies Not Involving Abnormal Sex-Chromosome Constitutions: Among the 22 Mongoloids so far studied cytologically in this and other laboratories, no departure from the trisomy of the smallest chromosome, discovered by LeJeune, Gauthier, and Turpin (1959a and b) has been found (Table 4). Figure 16 depicts the idiogram of a male Mongoloid examined by us.

Fifteen other human genetic and/or developmental defects fully or provisionally analyzed for somatic chromosome constitution include:

(a) One case of *polydysspondylism* with 45 chromosomes in which 2 autosomes had become attached to one another by translocation (Turpin et al., 1959).

(b) Fourteen anomalies in all of which the modal chromosome number was 46 and the gross chromosomal morphology appeared normal, viz.: one case each of *Marfan's syndrome, phenyl ketonuria, female pseudo-hermaphroditism, Gaucher's disease* (Tjio, Puck, and Robinson, 1959); four *anencephalic fetuses* (Harnden, Briggs, and Stewart, 1959); one case each of *epiloia, Laurence-Moon-Biedl syndrome, neurofibromatosis, arachnodactyly, osteogenesis imperfecta, achondroplasia* (Harnden, quoted in anonymous Lancet editorial, 1959), one *haemophilic* "girl" with male sex-chromatin pattern (Nilsson et al., 1959);

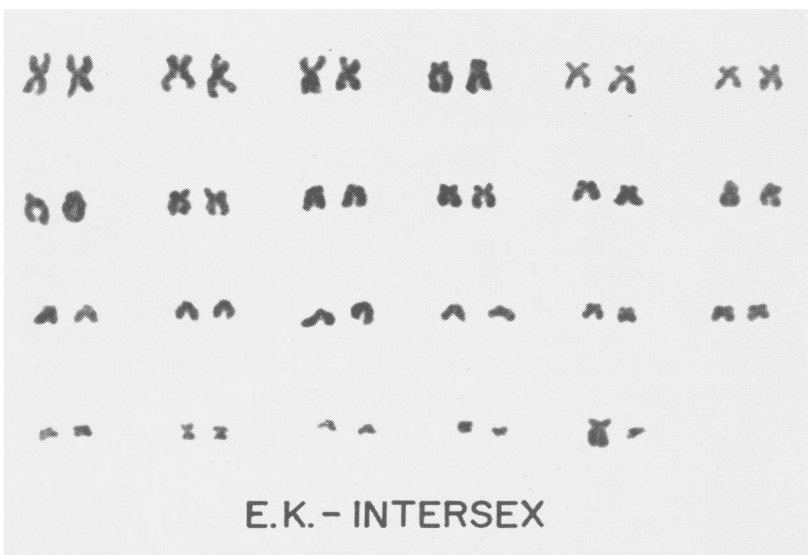

FIG. 15—Diploid idiogram (2n = 46) from marrow of E.K., an intersex. The fifth chromosome pair in the bottom row is interpreted as XY. (× 2000).

TABLE 4. SUMMARY OF CHROMOSOME CONSTITUTION IN 22 CASES OF MONGOLISM

Number of cases	Sex	Characteristic chromosome number	Idiogram details	Reference
10	6♂, 4♀	47	3 of smallest A*	Lejeune et al., 1959a,b
6	3♂, 3♀	47	3 of smallest A	Jacobs et al., 1959a
1	♂(Klinefelter's)	48	XXY + 3 smallest A	Ford et al., 1959a
3	2♂, 1♀	47	3 of smallest A	Böök et al., 1959
2	2♂	47	3 of smallest A	Present report

* A = Autosome

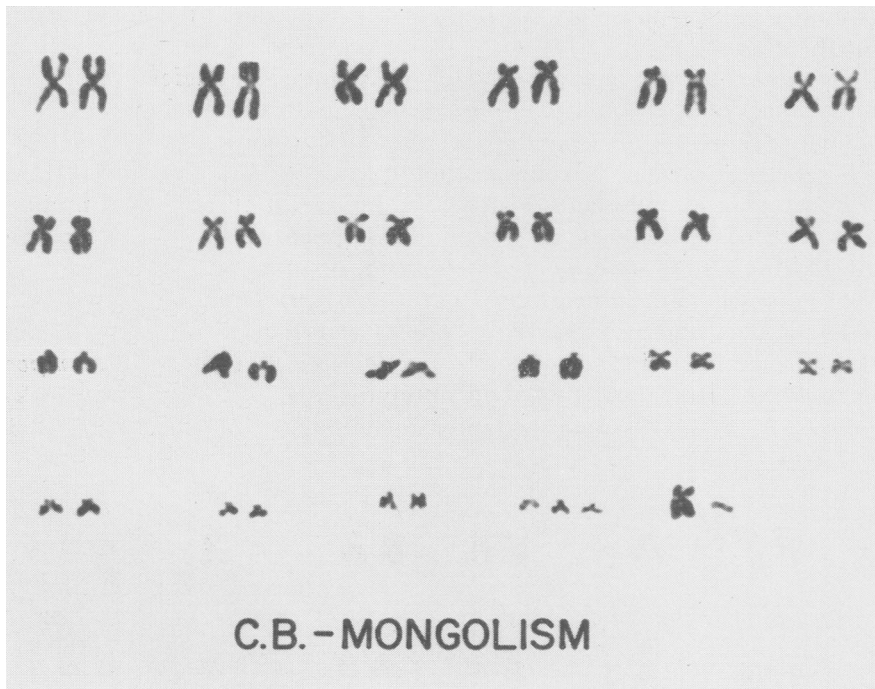

FIG. 16—Hyperdiploid idiogram (2n + 1 = 47) from marrow of C.B., a male mongoloid. The cell is trisomic for the smallest autosome, fourth group in the bottom row, to the left of the XY. (× 2000).

two cases of *Lowe's syndrome*, and one patient with *pseudohypoparathyroidism* (present report, Table 1, lines 12 and 13).

3. Karyotypic changes in leukemia

To establish the degree of aneuploidy compatible with viability of human marrow cells, our data on 7 leukemias and 1 aplastic anemia (Table 5) are here briefly discussed in relation to all other available findings in short-term tissue cultures of leukemic marrows and blood (Table 6).

After Nowell, Hungerford and Brooks (1958) had reported normal diploid chromosome constitutions in blood cultures from 3 cases with acute granulocytic leukemia, Ford, Jacobs and Lajtha (1958) reported observations of both numerical and structural chromosome anomalies in a "blast-cell leukemia". Baikie et al. (1959, 1960a, and b) examined a larger series. They found karyotypic changes in the stem-cell types of 4 among 5 acute leukemias, but normal chromosome conditions in 5 chronic myeloid leukemias, 1 chronic lymphatic leukemia, and 2 cases of myelomatosis. In considering the relationship of chromosomal anomalies to the pathogenesis of acute leukemia, they favored the view that "acute leukemias are the result of changes in the genetic material of the cell", whereas chronic leukemogenesis may involve "separate mechanisms of induction".

Ford and Mole (1959) questioned the latter assumption, pointing out the possibility that the normal metaphases seen in marrow-cultures from patients with chronic leukemia "were really normal marrow cells which must inevitably be found to some extent in aspirated bone marrow samples". This highly justified criticism applies, in our opinion, to neoplastic karyology by short-term tissue culture methods in general. Goldstein and Hauschka (unpublished) have counted chromosomes in human ovarian ascitic carcinoma and other tumors *before* (highly aneuploid) and *after* (mostly 46) brief tissue culture. We concluded that, even in relatively complete tissue culture media, the more exacting nutritional dependence of aneuploid neoplastic cells may enable normal host cells to overgrow them *in vitro,* or at least resume cellular multiplication before the unbalanced neoplastic karyotypes gain growth momentum.

For this reason, all our leukemic marrows were fixed immediately after procurement from the patient. The findings in Tables 5 and 6 (lines 8, 9) indicate a wider karyotypic variability and more frequent aneuploid modality among both chronic and acute leukemias than observed by other investigators.

A typical hyperdiploid idiogram from a chronic myelocytic leukemia (Table 5, line 7) is pictured in Figure 17. After matching 21 pairs, 6 chromosomes, probably including the X and Y, are left in the bottom row. Two of these are unusually small and 2 may be autosomes for which this set is trisomic.

The 5 chromosomes in the bottom row of Figure 18 represent a similar aneuploid excess over the tetraploid number of 92 in the modal idiogram of an entirely hypertetraploid, acute lymphoblastic leukemia.

Chromosomal imbalance of a degree that would probably be lethal in embryogenesis is thus not only viable on the cellular level, but may sometimes be contributory to the neoplastic growth advantage.

Since all the data summarized in Table 6 are no doubt valid, the discrepancy between our findings and, especially, those of Bayreuther (1960) raises the question: which cells are mitotically most active in fresh marrow biopsies, as against short-term marrow cultures in a relatively incomplete medium? Cytologic results obtained by both methods for each patient, and uncomplicated by transfusion, X-ray treatment or chemotherapy, should help settle this question.

Bayreuther (1960) completely discounts the carcinogenic potential of aneuploidy and considers the karyotypic irregularities observed in leukemias and

TABLE 5. CHROMOSOME CONSTITUTION OF LEUKEMIC, ANEMIC AND NORMAL HUMAN MARROWS
(based on 537 exact counts)

Case and diagnosis	Prior treatment	Marrow findings	Distribution of exact chromosome counts ←Diploid range→ ←Tetraploid Range→																Total counts	% Cells with 46 chromosomes
			<44	44	45	46	47	48	49–53	54–91	92	93	94	95	96	97	98	99	>99	
D.M., 3½ yr. ♂ Acute lymphoblastic leukemia in complete remission	Steroids, dichloromethotrexate	Normal, normoblasts slightly increased	3	3	1	*46*	1	—	—	—	—	—	—	—	—	—	—	—	—	54	85
P.B., 12 yr. ♂ Acute lymphoblastic leukemia	Steroids, X-ray, transfusion	No megakaryocytes, over 95% lymphoblasts	—	—	—	—	—	—	—	—	—	—	1	—	3	*9*	4	4	2	23	0
R.D., 4 yr. ♂ Acute lymphoblastic leukemia	None	Few megakaryocytes, 75% lymphoblasts	—	1	3	8	*14*	3	2	—	—	—	—	—	—	—	—	—	—	31	26
R.A., 64 yr. ♀ Acute myeloblastic leukemia	Transfusion	Hypocellular, but many myeloblasts	—	2	*8*	4	—	—	—	—	—	—	—	—	—	—	—	—	—	14	29
A.L., 58 yr. ♂ Acute myeloblastic leukemia in relapse	Transfusion, steroids, amidazolethioguanidine	Rare megakaryocytes, few normoblasts, 76% myeloblasts	2	4	6	*36*	11	9	2	—	—	—	—	—	—	—	—	—	—	70	51
E.D., 71 yr. ♂ Chronic lymphocytic leukemia	None	Few megakaryocytes, >90% lymphocytes	—	—	3	4	—	—	—	—	4	—	—	—	—	—	—	—	—	11	36

J.McG., 57 yr. ♂ Chronic myelocytic leukemia in blastic phase	Transfusion, myleran colcemide	Increased myeloblasts and promyelocytes, few megakaryocytes and normoblasts	—	—	—	2	2	*12*	3	—	—	—	—	—	—	—	—	—	—	19	10
A.A., 24 yr. ♂ Aplastic anemia	None	Very hypocellular, mostly plasma cells, lymphocytes and histiocytes	—	—	—	1	*6*	1	—	—	—	—	—	—	—	—	—	—	—	8	12
10 Normal controls (5♂, 5♀)	None	Normal	—	8	18	*276*	5	—	—	—	(~4% polyploids, none counted)							—	—	307	90

TABLE 6. SUMMARY OF CHROMOSOME CONSTITUTION IN 35 HUMAN LEUKEMIAS

Number of cases	Diagnosis	Chromosome numbers determined in	Modal karyotype shows numerical and/or structural abnormalities	Reference
3	2 blast cell, 1 lymphatic	Marrow culture	1/3	Ford et al., 1958
5	Acute myelocytic	Marrow culture	4/5	Baikie et al., 1959 and
8	Chronic myeloid & lymphatic	" "	0/8	Baikie et al., 1960a,b
2	Acute myelocytic	Blood culture	0/2	Nowell & Hungerford,
2	Chronic myelocytic	" "	2/2	1960
5	Acute myeloid & lymphatic	Marrow culture	0/5	Bayreuther, 1960
3	Chronic myeloid & lymphatic	" "	0/3	" "
5	Acute myeloid & lymphatic	Marrow directly from patient	3/5	Present report
2	Chronic myeloid & lymphatic	Marrow directly from patient	2/2	" "

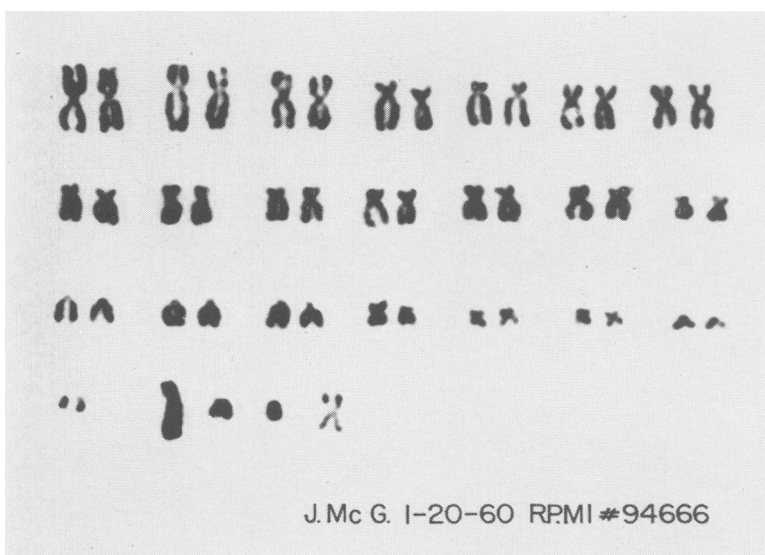

FIG. 17—Hyperdiploid leukemic idiogram (stem-cell type with 48 chromosomes) from marrow of J.McG., a male patient with chronic myelocytic leukemia in blastic phase. (× 1600).

other primary tumors as mere epiphenomena in the wake of a more fundamental cellular alteration. A reasonable alternative to this extreme viewpoint would be to accept chromosomal imbalance as one among several genetic and non-genetic mechanisms whereby a cell can achieve neoplastic autonomy. That aneuploid

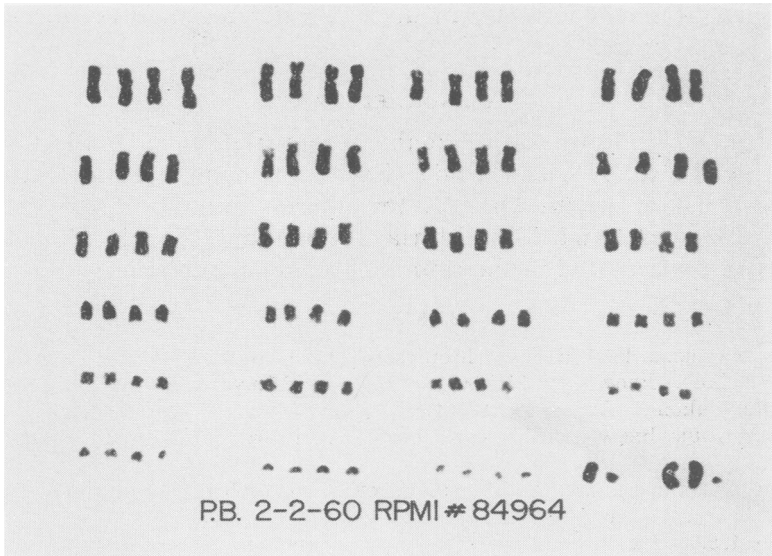

FIG. 18—Hypertetraploid leukemic idiogram (stem-cell type with 97 chromosomes) from marrow of P.B., a male patient with acute lymphoblastic leukemia. (× 1300).

cells and organisms may function within the precinct of relatively normal growth is, however, self-evident from the somatic karytoypes in some human developmental disorders.

SUMMARY

The chromosome pattern (number, morphology and frequency of chromosomal abnormalities) was examined in the marrows from 10 normal human subjects, 24 patients with developmental anomalies, 1 aplastic anemia, and 7 leukemias. In the 10 normal marrows, the chromosome number of 46 occurred with a frequency of 90% ± 1.7; about 0.3% true somatic pairing in diploids, and up to 4% polyploids were seen.

Essentially, our findings for ovarian agenesis, Klinefelter's syndrome and Mongolism agree with earlier published work. However, a larger-than-expected incidence of mosaicism, including karyotypes tri- or mono-somic for small as well as large autosomes, has come to light. Heteropycnosis and allocycly of the X-chromosome, not previously noticed in the human idiogram, are especially pronounced in Turner's syndrome. A probably triple-X, nine-year-old "super-female" with precocious puberty, and a case of intersex with a male chromosome complement of 46 (XY) are described.

In 7 acute and chronic leukemic marrows, fixed immediately after sternal aspiration (rather than after temporary growth *in vitro*), aneuploidy was far more frequent than in normal controls. One leukemia was entirely hypertetraploid with a mode at 97, two had hyperdiploid modes at 47 and 48, and one had a hypodiploid cell population mode at 45 chromosomes.

All available chromosome data on human marrows in developmental and blood

disorders are tabulated for convenient cross-reference, and are discussed in relation to our findings.

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