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Quinacrine Mustard Fluorescence of Human Chromosomes: Characterization of Unusual Translocations

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Of the human metaphase chromosomes, only four autosomal pairs (nos. 1, 2, 3, and 16) and frequently the Y can be identified individually by conventional staining techniques. Autoradiography with tritiated thymidine is useful mainly in differentiating among B-, D-, and E-group chromosomes and in detecting the late-replicating X in the C group and the Y in the G group [1, 2]. Individual chromosomes in the C and F groups cannot be recognized, with the exception of no. 9, which often reveals a secondary constriction on the long arms close to the centromere [3]. Thus, clinical syndromes associated with structural abnormalities of the remaining C- and F-group chromosomes have not yet been defined.

However, after staining with intercalating fluorescent agents, especially quinacrine mustard (QM), each individual chromosome pair can be identified by its characteristic pattern of bright and dark bands visible in the fluorescence microscope [4, 5]. The differential binding of QM is presumably based upon the amount of DNA present in a certain chromosome region and upon its accessibility to the stain as determined by the presence of DNA-binding proteins [6]. Specific covalent binding of the mustard group to guanine residues of the DNA does not cause significant differences in fluorescence pattern compared with staining with quinacrine dihydrochloride, which lacks an alkylating group; but the bright bands are much more pronounced in QM-stained preparations [7]. Initial work with fluorochrome staining focused on identification of the intensely fluorescing area on the Y chromosome in interphase nuclei [8], metaphase spreads [9], and spermatocytes [10]. Application to metaphases containing chromosomal abnormalities established the following facts: (1) the extra chromosome in Down's syndrome, either as trisomy 21 or in 21/21 or D/21 translocations, was identified as the smaller and more intensely fluorescent chromosome in the G group [11]; (2) the Philadelphia chromosome (Ph') in chronic myelogenous leukemia was shown to be a deleted no. 22 [11, 12]; (3) correlation of specific band patterns of B-group chromosomes with

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nos. 4 and 5 was accomplished by comparison with autoradiographic studies of normal individuals and one patient with cri du chat syndrome [13]; and (4) identification of D-group chromosomes involved in trisomies and translocations was consistent with results obtained by autoradiography [14].

In the present study, QM staining was applied to metaphases from 16 individuals carrying various translocations in balanced or unbalanced form. Some of the chromosomal rearrangements had been misinterpreted or unrecognized in previous routine cytogenetic studies. By fluorochrome staining, all the affected chromosomes were specifically identified. Furthermore, the amount of translocated material was estimated by determining the breakpoints within the chromosomes visually as well as by analyzing photoelectrical recordings of the fluorescent patterns. The significance of the technique for more accurate genetic diagnosis and counseling is emphasized.

METHODS

Patients

The patients selected for fluorescent chromosome analysis had been studied previously in our laboratory with routine and, in some cases, autoradiographic techniques. Detailed clinical and genetic findings have either already been reported (cases 2 [15] and 11 [16]) or will be published separately. Case 2 was detected during a survey of psychiatric patients. In all other instances, a phenotypically abnormal mentally retarded child was referred for chromosomal analysis. In cases 5–11, an apparently balanced translocation, which was found in a phenotypically normal parent, gave rise to an unbalanced state in one or more offspring. In cases 1–4, an apparently balanced, possibly reciprocal translocation arose de novo, being present only in the propositi. The patients are designated by numbers derived from their initials and their birthdates as suggested by the Chicago Conference [17].

Preparation and Staining of Slides

Slides were made from routine blood cultures. Flame and air drying techniques were compared and yielded equally good results. The highest degree of differential QM binding was obtained with recently prepared slides. When slides were more than 2 months old, results were generally less satisfactory. After staining 1-year-old slides, the Y chromosome could still be identified by its more intense fluorescence, but the band pattern on the other chromosomes was not sufficiently distinct to allow complete and reliable karyotyping.

Slides were stained for 20 min with QM in McIlvaine's disphosphate citric acid buffer (*p*H 7) at a concentration of 50 μg/milliliter [4]. After three rinses in buffer, the slides were mounted in buffer and sealed with nail polish.

Photomicroscopy

A Zeiss standard fluorescence microscope was used with transmitted light, a BG 12 exciter filter, oiled darkfield condenser, and barrier filters 53 and 44. Metaphases consisting of moderately elongated chromosomes without overlaps were photographed through a 100× planapochromat objective with an iris. Kodak Tri X 35mm film was used and was developed in Microdol X. Prints were made on Agfa-Gevaert paper no. 4.

Preparation of the Karyotypes

Karyotypes were arranged according to Caspersson's most recently published standard karyotype [18], which is based on correlation with autoradiography and on centromere

index and relative chromosome length. These authors have inverted chromosome 1 compared with their earlier publications, the present arrangement being in agreement with the Chicago Conference 1966 [17].

Microdensitometer Tracing

Transparent images of printed chromosomes were obtained with an MP 3 Polaroid camera in a cell-to-transparency enlargement of approximately 1,500×. They were scanned on a Joyce Loebl microdensitometer by moving the scanning slit (width: 2.8 mm) broadside down the axis of the chromosome images covering both chromatids. In the resulting distribution curves of fluorescent intensity, maxima correspond to bright bands on the chromosomes.

RESULTS

A brief summary of the clinical findings in the 11 patients is given in table 1, together with the interpretation of the karyotypes from routine and autoradiographic analyses and from fluorescent studies. Specific comments will cover only the interpretation of the fluorescent karyotypes.

Case 1: EC 082963

The conventional karyotype shows an abnormal chromosome in the D group (fig. 1*a*). Autoradiographic analysis was not conclusive in identifying this Dp+q+. Examination of nine fluorescent karyotypes (fig. 1*b*) reveals only one normal member of pairs 1 and 13. The other no. 1 is missing two-thirds of its short arms. The missing fragment appears to be translocated to the short arms of a no. 13. The resulting metacentric (13p+) shows the fluorescent pattern of the long arms of a no. 13 in one set of its arms and that of the distal short arms of a no. 1 in the other set and is thus clearly distinguishable from pair 3, which has the same size and shape. In conventionally stained karyotypes, this 13p+ was paired with the normal no. 1, resulting in a nonhomologous pair thought to be due to a difference in contraction. The deleted no. 1 was interpreted as an abnormal D (Dp+q+) (fig. 1). In addition, one chromosome 22 was found to have no short arms. Whether this missing material is involved in a translocation or has been lost could not be determined. The fluorescence distribution profiles of the translocation chromosomes and their normal homologs, as shown in figure 2*a*, confirm the visual analysis and allow determination of the breakpoint on no. 1. The interpretation of the patient's karyotype is expressed as: 46,XX,t(1p−;13p+),22p−.

Case 2: LS 020951

Routine chromosome analysis was interpreted as showing a balanced t(Cq−; Gp+). In addition, one G appeared to have elongated long arms. Fluorochrome staining of 10 metaphases reveals a chromosome 10 missing two-thirds of its long arms and a chromosome 22 with material added to its short arms, suggesting a t(10q−;22p+) (fig. 3*a*, *b*). Chromosome 10 is one of the smallest in the C group and exhibits three bright bands on the long arms, the most proximal one being the brightest. The break appears to have occurred just distal to this intensely fluorescent band, as is demonstrated accurately by the intensity profiles (fig. 2*b*). The G with

TABLE 1

SUMMARY OF CLINICAL AND KARYOTYPIC FINDINGS

CASE NO., SEX, AND AGE	CLINICAL FINDINGS	KARYOTYPE ANALYSIS BASED ON			COMMENT
		Routine Staining	Autoradiography (RAG)	QM Staining	
1) EC 082963, female, 7 years	Pierre Robin syndrome: micrognathia, macroglossia, cleft palate. Severe mental retardation, spastic quadriplegia [19]	46,XX,Dp+q+ translocation of unknown material onto a D-group chromosome	Inconclusive	46,XX,t(1p−; 13p+),22p−	Correct identification of presumed Dp+q+ as 1p−; apparently balanced translocation; identification of 22p−
2) LS 020951, female, 20 years	Manic depressive psychosis, mild mental retardation, low set ears, short upper lip [15, 20]	46,XX,t(Cq−; Gp+),Gq+	Uninformative	46,XX,t(10q−; 22p+),21q+	Accurate identification of balanced translocation and of 21q+
3) LB 062260, male, 11 years	Moderate mental retardation, large eyes and nose, prominent nasal bridge, hypoplastic maxilla, inguinal and umbilical hernias	46,XY,t(Cq−; Cq+)	Not done	46,XY,t(6q+; 12q−)	Probably balanced translocation; no apparent loss of chromosomal material
4) LL 100668, female, 2 years	Psychomotor and growth retardation, slight hypertelorism and antimongoloid slant, congenital corneal leukoma and optic atrophy, decreased corneal sensitivity	46,XX,Eq−; minor cell line: 46,XX, Bq+,Eq−; suspected: 46,XX,t (Bq+;Eq−)	46,XX,18q−; no information on minor cell line	Three different cell lines: 46, XX,t(11p+; 17q−); 45, XX,17−; 11p+; 46, XX,t(11p+; 17q−),5q+	Detection of mosaicism; identification of 11p+ in C group; identification of no. 17 as deleted E instead of no. 18 as suggested by RAG

TABLE 1 (*Continued*)

Case No., Sex, and Age	Clinical Findings	Karyotype Analysis Based on: Routine Staining	Autoradiography (RAG)	QM Staining	Comment
5) MS 011467, male, 4 years	Moderate mental retardation, prominent occipital and parietal regions of skull, small palpebral fissures, epicanthal folds, limitation of motion in neck and hips, hypoplastic external genitalia	46,XY,Fq+; partial trisomy for a B	46,XY,Fq+; partial trisomy for no. 5	46,XY,20q+ mat; trisomy for distal half of long arms of no. 4	Correct identification of Bq− as no. 4 instead of no. 5 and Fq+ as no. 20; confirmation of the assumed involvement of the long arms of no. 20
Mother ...	Normal	46,XX,t(Bq−; Fq+)	46,XX,t(5q−; Fq+)	46,XX,t(4q−; 20q+)	
6) KW 061268, female, 3 years	Severe developmental and growth retardation, ptosis, microphthalmia, depressed nasal bridge, cleft palate, micrognathia, low-set malformed ears	46,XX,Dp+	46,XX,15p+	46,XX,15p+ mat; trisomy for distal two-thirds of long arms of no. 10	Identification of Cq− as no. 10; confirmation of 15p+ identified by RAG; unequivocal differentiation of 15p+ from C group
Mother ...	Normal	46,XX,t(Cq−; Dp+)	46,XX,t(Cq−; 15p+)	46,XX,t(10q−; 15p+)	
7) RQ 052561, male, 10 years	Profound mental retardation, delayed growth, hypotonic musculature, narrow head, frontal bossing, endophthalmus, microcornea, horizontal nystagmus	46,XY, 2q+	Not done	46,XY,2q+; trisomy for short arms of no. 11	Identification of C?− as a no. 11 with deleted short arms
Father ...	Normal	46,XY,2q+,C−, D+; probably: 46,XY,t(2q+; C?−)	Not done	46,XY,t(2q+; 11p−)	

TABLE 1 (*Continued*)

Case No., Sex, and Age	Clinical Findings	Karyotype Analysis Based on: Routine Staining	Autoradiography (RAG)	QM Staining	Comment
8) SD 030170, female, 1 year	Retarded psychomotor development, microcephaly, prominent glabella, wide prominent nasal bridge, epicanthal folds, hypoplastic nails	47,XX,mar+	47,XX,(18q−)+; trisomy for 18q−	47,XX(18q−)+mat; trisomy for 18q−	Identification of no. 9 involved in balanced translocation in the mother; confirmed RAG finding of 18q−
Mother ...	Normal	46,XX,t(Cp+; Eq−)/47,XX, Cp+;mar+	46,XX,t(Cp+; 18 q−)	46,XX,t(9p+; 18q−)	
9) CJ 121468, female, died at 5 weeks ..	Micrognathia, macroglossia, incomplete cleft palate, dislocated hips, atrial septal defect, agenesis of corpus callosum, one kidney and one fallopian tube	46,XX,Bq+	Not done	Not done; trisomy for distal third of long arms of no. 11	Identification of Cq− as no. 11; confirmation of 4q+ identified by RAG; diagnosis of proposita: 46,XX,4q+mat
Maternal aunt ...	Normal	46,XX,t(Bq+; Cq−)	46,XX,t(4q+; Cq−)	46,XX,t(4q+; 11q−)	

TABLE 1 (*Continued*)

Case No., Sex, and Age	Clinical Findings	Karyotype Analysis Based on: Routine Staining	Autoradiography (RAG)	QM Staining	Comment
10) KD 071668, female, died during first year	Premature birth, low birth weight, prominent forehead, flat nasal bridge, generalized hypotonia, large patent ductus arteriosus	46,XX,Gq+	Not done	Not done; trisomy for distal third of long arms of no. 16	Confirmation of suspected balanced translocation in father; identification of 22q+; diagnosis of propositа: 46,XX,22q+pat
Father ...	Normal	46,XY, Gq+; nonhomology of pair 16, possibly 46,XY,t(16q−; Gq+)	Not done	46,XY,t(16q−; 22q+)	
11) LR 082262, female, 9 years	Moderate mental retardation, flat occiput, round face, thin nose, wide nasal bridge, mongoloid slant, widely spaced teeth, micrognathia, hyperextensible joints	Initially: 46,XX, normal; later: 46,XX,Dq+	46,XX,13q+	46,XX,13q+ mat; trisomy for short arms of no. 20	Confirmation of RAG finding of 13q+; identification of translocated fragment as short arms of no. 20
Sister	Same as LR	46,XX,Dq+	46,XX,13q+	Not done	
Brother ..	Same as LR [15]	46,XY,Dq+	46,XY,13q+	Not done	
Mother ...	Normal	46,XX,t(Dq+; F?−)	46,XX,t(13q+; F?−)	46,XX,t(13q+; 20p−)	

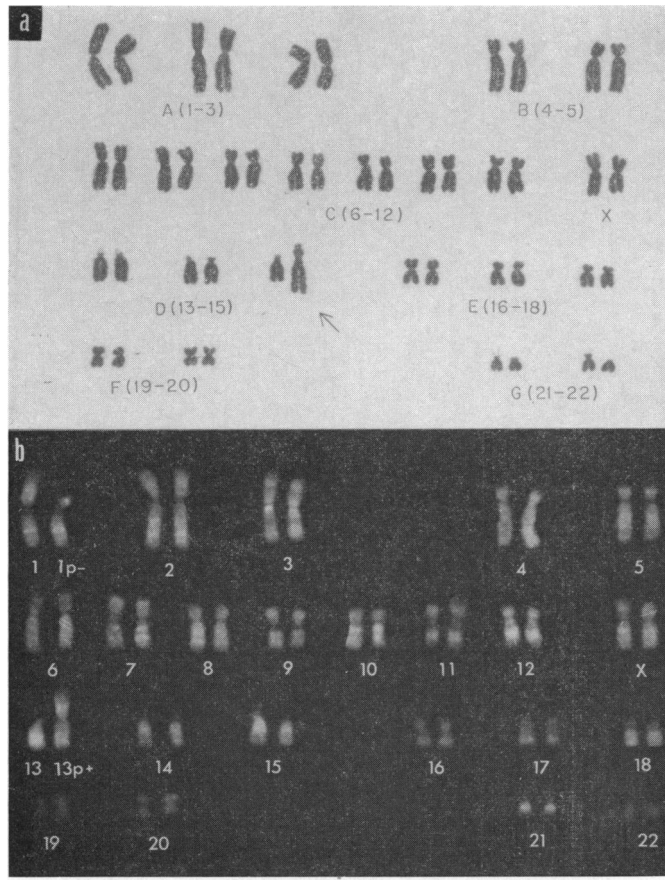

FIG. 1.—Case 1, EC: (*a*) Giemsa-stained karyotype; arrow indicates abnormal chromosome Dp+q+; note nonhomology of pair 1. (*b*) QM-stained karyotype shows t(1p−;13p+).

elongated long arms is identified as a no. 21 with less intensely fluorescing material added distal to the usual bright band. This chromosome is also present in other family members and therefore is probably independent of this patient's de novo balanced reciprocal translocation. The complete cytogenetic diagnosis is written as: 46,XX,t(10q−;22p+),21q+.

Case 3: LB 062260

Two morphologically abnormal C-group chromosomes suggest the presence of a t(Cq+;Cq−). After examination of five QM-stained karyotypes, the elongated C is identified as a no. 6 (6q+) by the characteristic dark band in the middle of the short arms, and the deleted C is identified as a no. 12, which appears to be missing the distal half of its long arms (fig. 3*c*, *d*). The amount of translocated material can be estimated by comparing the band patterns and intensity distribution curves of the normal and abnormal nos. 6 and 12 (fig. 2*c*). The point of rejoining on 6q+ is

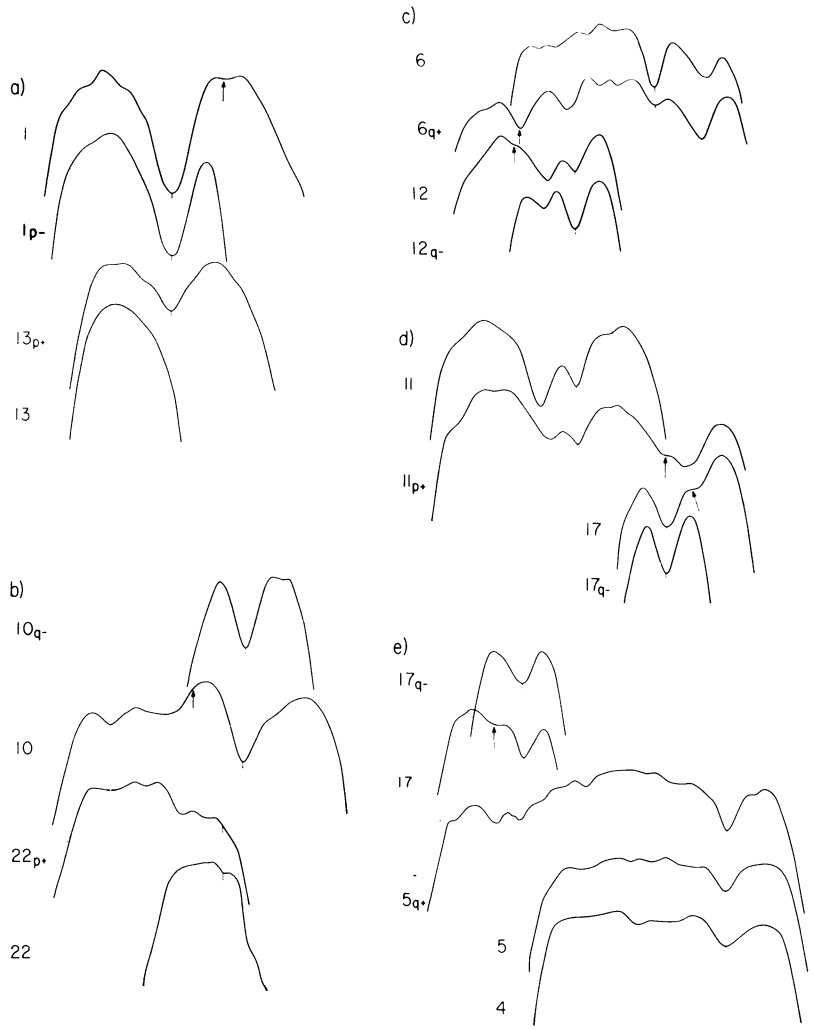

FIG. 2.—Fluorescent intensity profiles of chromosomes from (*a*) case 1, EC; (*b*) case 2, LS; (*c*) case 3, LB; (*d*) case 4, LL, major cell line showing t(11p+;17q—); (*e*) case 4, LL, minor cell line containing 5q+ and 17q—. Centromeres are indicated by vertical lines through curves. Arrows point to probable breakpoints. Long arms are represented on the left side of the profiles, short arms on the right, except in (*b*) and (*d*), where 22p+ and 22, and 17 and 17q— have been inverted to facilitate comparison of the curves.

located in the area proximal to the bright band, since the latter is not present on the normal no. 6. If a reciprocal transposition of material from the distal long arms of the no. 6 to the deleted long arms of the no. 12 has occurred, the piece is too small to be seen by this technique. The most likely interpretation is that of a probably balanced translocation between the long arms of chromosomes 6 and 12: 46,XY,t(6q+;12q—).

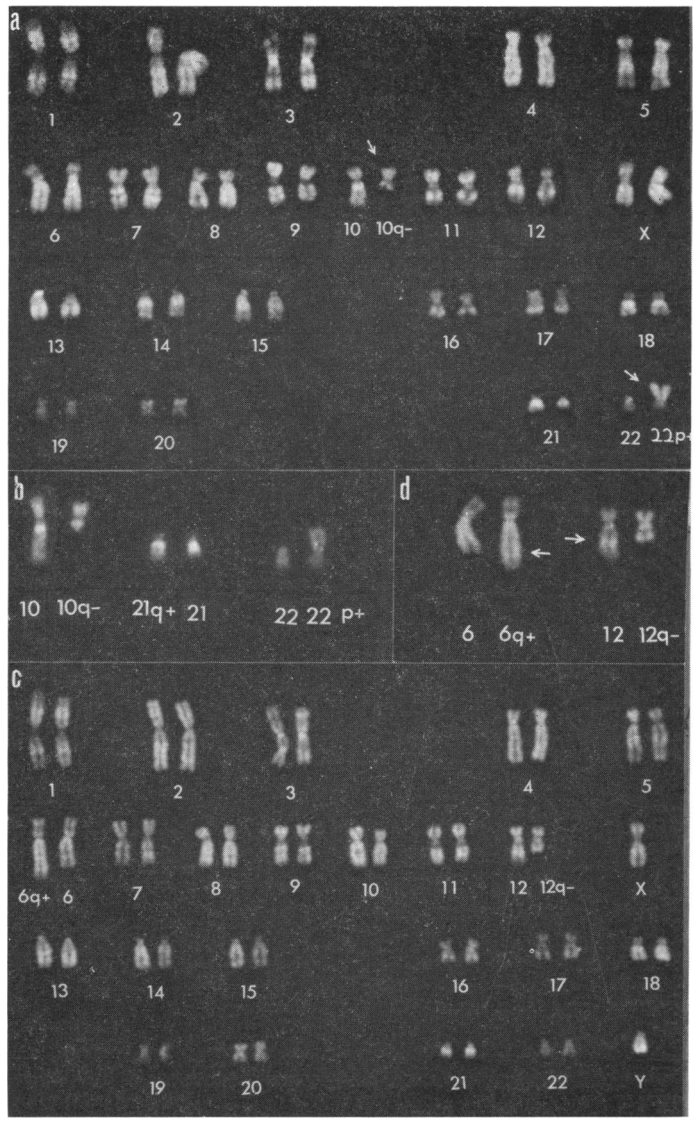

FIG. 3.—(*a*) Case 2, LS, full fluorescent karyotype; arrows indicate abnormal chromosomes. (*b*) Partial karyotype of LS shows the break in no. 10 just distal to the bright band; note 21q+. (*c*) Case 3, LB, full fluorescent karyotype. (*d*) Partial karyotype of LB; arrows indicate probable points of breakage and rejoining.

Case 4: LL 100668

On QM-stained slides from two different lymphocyte cultures, 50 metaphases were examined and 14 karyotyped. Mosaicism for three different cell lines was found. The major cell line (80%–90%) has 46 chromosomes, including two no. 18s, one no. 17, and one metacentric smaller than an F, which most likely represents a

no. 17 missing about two-thirds of the long arms. In addition, one member of pair 11 is replaced by a larger submetacentric whose fluorescent pattern is consistent with its being a no. 11 with material translocated to the short arms (fig. 4*a*). This

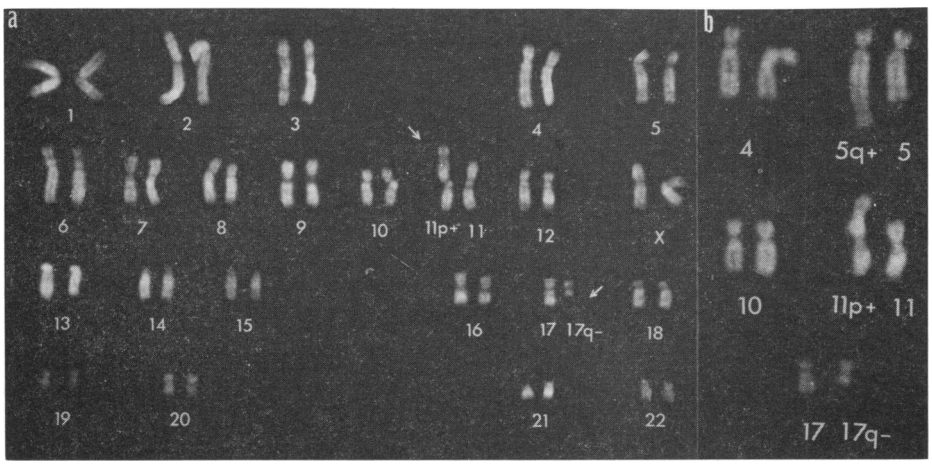

FIG. 4.—Case 4, LL: (*a*) Fluorescent karyotype of major cell line; arrows indicate the translocated chromosomes. (*b*) Partial karyotype of minor cell line containing t(11p+;17q—) and an elongated no. 5 (5q+).

11p+ had not been identified by routine and autoradiographic techniques; it had been placed upside down as the largest chromosome in the C group. Examination of the band pattern and the intensity distribution profiles (fig. 2*d*) supports the conclusion that this major cell line carries a balanced translocation between the long arms of a no. 17 and the short arms of a no. 11: 46,XX,t(11p+;17q—). One of the minor cell lines (2%–5%) contains 45 chromosomes, missing the small metacentric deleted no. 17. Four of the cells examined (5%–10%) represent another minor cell line with 46 chromosomes, including the balanced t(11p+;17q—), characterized by strikingly elongated long arms of a chromosome 5 (fig. 4*b*). The origin of this translocated material is not clear, and the complement appears to be normal otherwise. Intensity distribution profiles of the material translocated to the 11p+ and the 5q+ show similarities (fig. 2*e*).

Case 5: MS 011467

In routine chromosome analysis, an abnormal medium-sized subacrocentric chromosome was found instead of a normal F. In karyotypes from the mother, in addition, one B-group chromosome appears to be missing half of its long arms, suggesting a t(Bq—;F?+).

Seven fluorescent karyotypes prepared from the patient and eight from his mother (fig. 5*a*, *b*, *c*) as well as intensity distribution profiles of the affected chromosomes (fig. 6*a*, *b*) consistently show the following pattern: the abnormal subacrocentric present in both individuals represents a chromosome 20 that has intensely fluo-

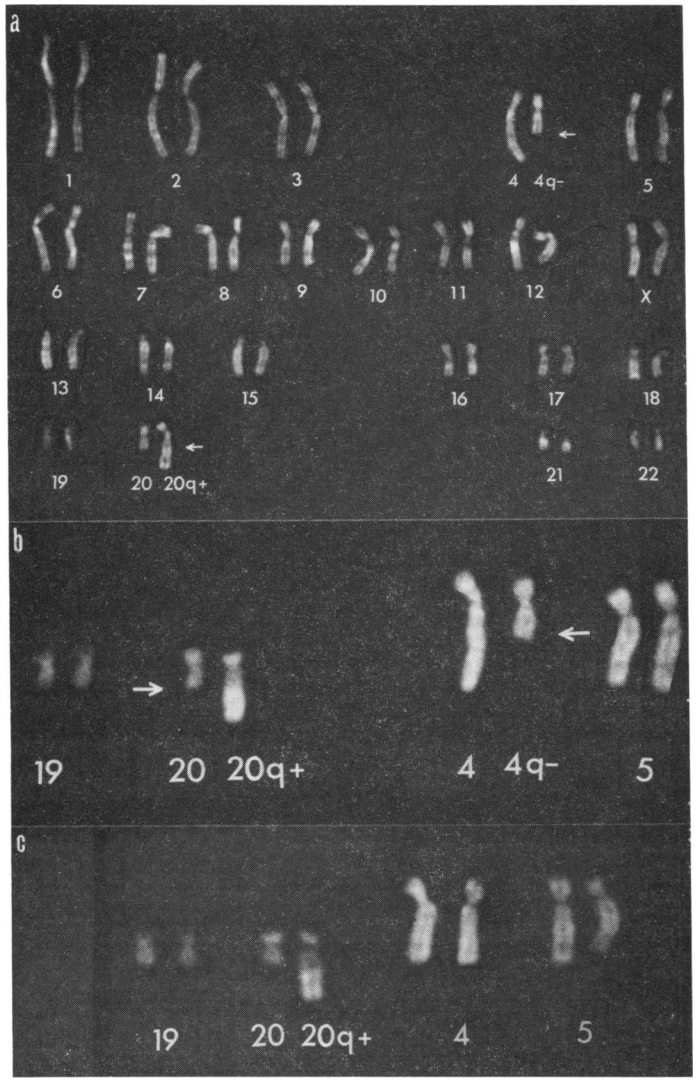

FIG. 5.—Case 5, MS: (*a*) Full and (*b*) partial fluorescent karyotype of MS's mother; arrows indicate chromosomes involved in the translocation. (*c*) Partial karyotype of propositus.

rescent material added to its long arms, which are distinguished from the short arms by weaker fluorescence. The pattern of the translocated material resembles that of the distal long arms of a no. 4 rather than of a no. 5. The mother's cells contain three normal B's, two of which show the pattern characteristic of a no. 5 and one which shows that of a no. 4. An extra submetacentric chromosome, intermediate in size between a C and a no. 16, has a fluorescent pattern consistent with the short arms and the proximal half of the long arms of a no. 4. The breakpoint in this translocation appears to lie between the second and third of the four evenly spaced bright

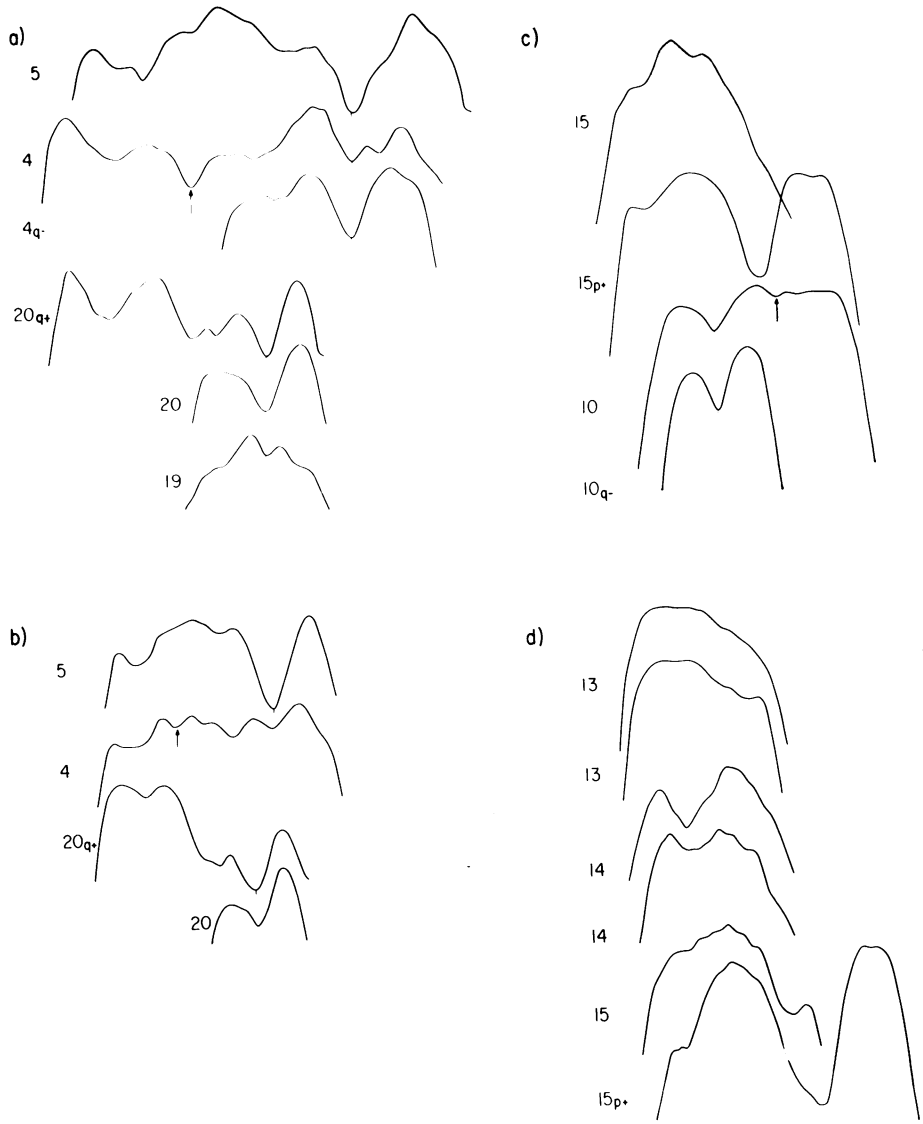

FIG. 6.—Fluorescent intensity distribution profiles of chromosomes from (*a*) mother of case 5, MS, showing t(4q−;20q+); (*b*) case 5, MS, with translocated 20q+; (*c*) mother of case 6, KW, showing t(10q−;15p+); (*d*) case 6, KW, with abnormal 15p+ compared with normal no. 15 and normal pairs 13 and 14; arrows show probable points of chromosome breakage. Long arms are represented on the left and short arms on the right side of the centromere (vertical lines) except in (*c*), where curves 10 and 10q+ are inverted to facilitate comparison between curves of translocated and normal chromosomes.

bands on the long arm of no. 4 with transposition of the two distal bands to the long arms of a no. 20 (fig. 5*a*, *b*). Since the mother's karyotype is 46,XX,t(4q—; 20q+), the patient can be diagnosed more specifically as being trisomic for the distal half of the long arms of chromosome 4: 46,XY,20q+mat.

Case 6: KW 061268

In routine karyotypes, a missing D is replaced by a large submetacentric distinguishable from a C-group chromosome by the lack of staining around the centromere in some preparations. Furthermore, in cells from the mother a regular C appears to be missing most of its long arms, suggesting a balanced t(Cq—;Dp+). In eight fluorescent karyotypes from the patient and five from the mother, the Dp+ can clearly be distinguished from the C group by the fluorescent pattern of its long arms, which is that of a no. 15 and does not resemble any normal C (figs. 6*d*, 7*a*, *b*). While the proposita's karyotypes contain a normal pair 10, one member of this pair has deleted long arms in the mother's cells (10q—). The characteristic bright band next to the centromere is retained and facilitates unequivocal differentiation between short and long arms in this small metacentric 10q—. Compared with case 2 (fig. 3*b*), the break in the long arms of chromosome no. 10 appears to have occurred more distally. (Compare also the intensity profiles in figs. 2*b* and 6*c*.) The mother carries an apparently balanced translocation between chromosomes 10 and 15: 46,XX,t(10q—;15p+). The proposita's abnormal phenotype is most likely related to her being trisomic for the distal two-thirds of the long arms of chromosome 10: 46,XX,15p+mat.

Case 7: RQ 052561

Chromosome studies from the blood show nonhomology of pair 2 with one member having markedly elongated long arms. This abnormality is also found in cells from the father, who, in addition, has a chromosome missing from the C group which is replaced by an acrocentric of the size of a D. In four QM-stained karyotypes from the father, the involved C is identified as a no. 11 which is missing the short arms almost completely (fig. 7*c*). This deleted chromosome is distinguished from the D's by its intense fluorescent pattern in the middle region, which is separated by a dark band from the bright area next to the centromere. The material transposed to the long arms of a no. 2 displays a pattern similar to the short arms of the normal no. 11 (figs. 7*c*, 8*a*). This is consistent with the cytogenetic diagnosis, 46,XY, t(2q+;11p—), in an apparently balanced state. Five fluorescent karyotypes from the propositus contain a normal pair 11 and an identical 2q+. He is therefore trisomic for the short arms of chromosome 11: 46,XY,2q+pat.

Case 8: SD 030170

All cells cultured from blood and skin contain 47 chromosomes because of an extra metacentric smaller than an F. Chromosome analysis on the parents reveals that the mother carries a balanced t(Cp+;Eq—) characterized by an almost metacentric C and an extra small metacentric marker chromosome, which is shown by

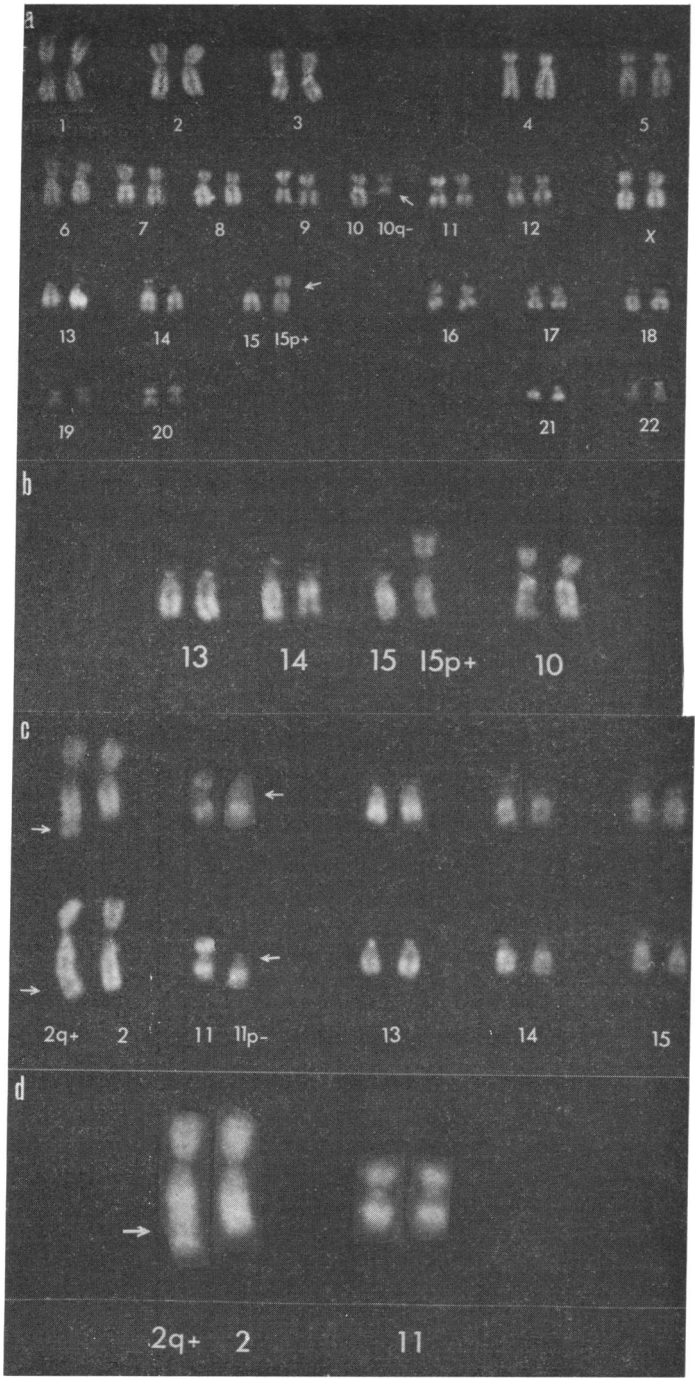

FIG. 7.—Case 6, KW: (*a*) Fluorescent karyotype of KW's mother with t(10q−;15p+). (*b*) Partial karyotype of proposita showing D group with 15p+ and normal pair 10. (*c*) Case 7, RQ: two partial fluorescent karyotypes from RQ's father; arrows indicate the translocated chromosomes; comparison with pairs 13, 14, and 15 allows one to distinguish 11p−. (*d*) Partial karyotype of case 7, RQ.

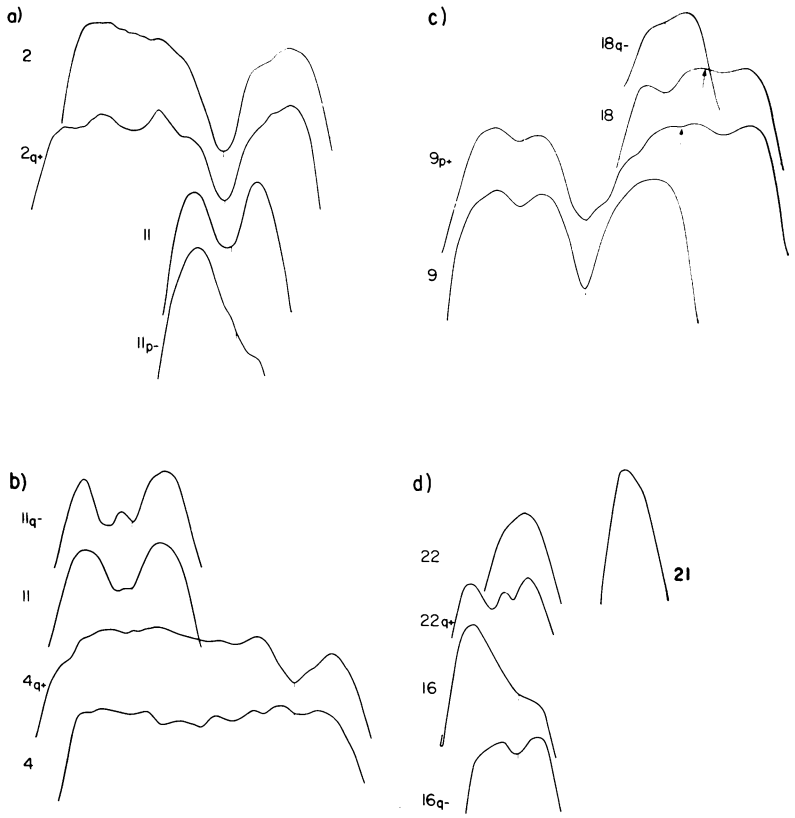

FIG. 8.—Fluorescent intensity distribution profiles of chromosomes from (*a*) father of case 7, RQ, with t(2q+;11p—); (*b*) aunt of case 9; CJ, showing t(4q+;11q—); (*c*) mother of case 8, SD, with balanced t(9p+;18q—); (*d*) father of case 10, KD, with t(16q—;22q+). Vertical lines cutting curves indicate positions of centromeres. Long arms are represented on the left side and short arms on the right side except for nos. 18q— and 18 in (*c*), which have been inverted.

autoradiography to be a deleted no. 18. A minor cell line with 47 chromosomes probably contains two normal no. 18s in addition to the translocation chromosomes; hence, the phenotypically normal mother is probably mosaic for partial trisomy 18. The presence of this cell line enabled her to transmit a normal no. 18 and the 18q— to her child in the same ovum. Other explanations are certainly possible but will not be further discussed here.

Six fluorescent karyotypes from the mother (fig. 9*a*) show 46 chromosomes with a small metacentric marker chromosome consisting of the short arms and part of the proximal bright band on the long arms of a no. 18. In addition, bright chromosomal material is transposed to the short arms of a no. 9, causing the short arms to exceed the long arms in length. The fluorescence pattern of this translocated material matches the pattern of the distal long arms of a no. 18 (figs. 8*c*, 9*a*). This probably balanced translocation is designated 46,XX,t(9p+;18q—). The QM-stained cells from the proposita contain the extra small metacentric 18q— (fig. 9*b*)

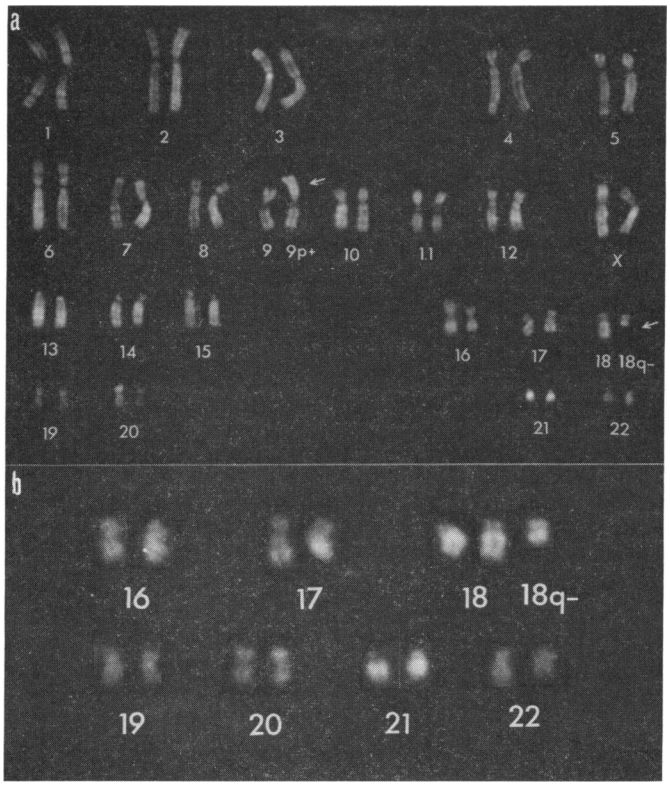

FIG. 9.—Case 8, SD: (*a*) Fluorescent karyotype of SD's mother; arrows indicate translocated chromosomes. (*b*) Partial karyotype from the proposita showing an extra small metacentric chromosome with the fluorescent pattern of an 18q—.

and normal pairs 9 and 18. She is therefore specifically characterized as being trisomic for the short arms and the proximal quarter of the long arms of chromosome 18: 47,XX,(18q—)+mat.

Case 9: CJ 121468

In routine analysis on the proposita, the only abnormal chromosome finding was a slight elongation of the long arms of a B-group chromosome. Subsequently, the mother and other members of her family were found to carry an apparently balanced t(Bq+;Cq—), based upon the finding of an elongated B present together with a small abnormally metacentric C.

Fluorochrome staining was carried out on four metaphases from a maternal aunt known to carry the translocation. One member of pair 11 is lacking the weakly fluorescent material distal to the bright band on its long arm. A similar amount of rather faintly staining material appears to be added to the long arm of a no. 4, whose distal terminal bright band appears fully preserved (figs. 8*b*, 10*a*). Apparently, a balanced translocation has taken place, resulting in the abnormal karyo-

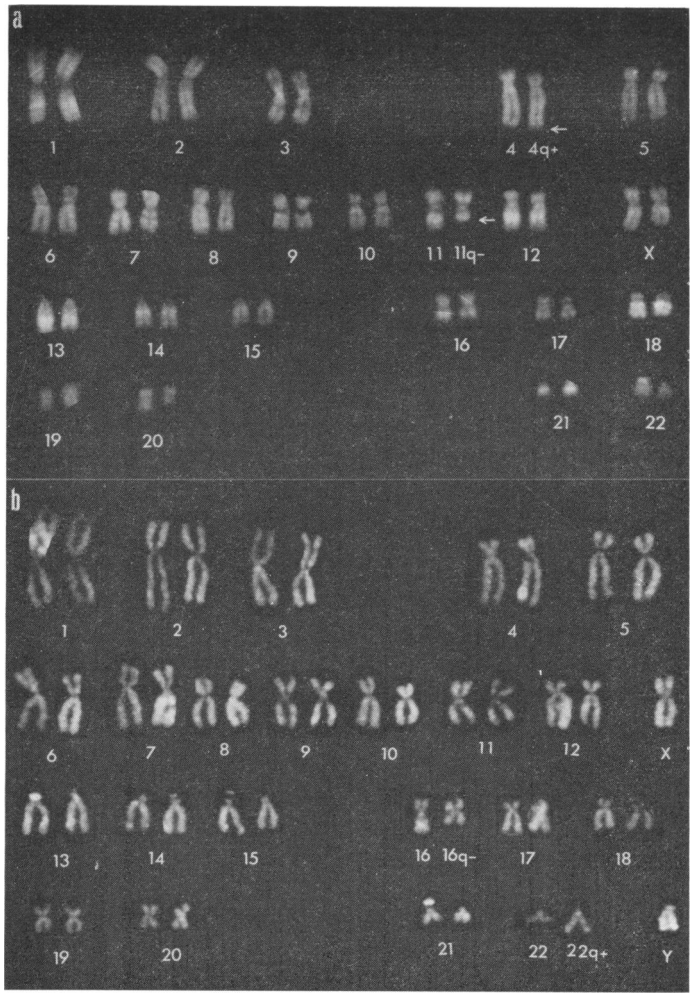

FIG. 10.—(*a*) Fluorescent karyotype of aunt of case 9, CJ, with t(4q+;11q—). (*b*) Fluorescent karyotype of father of case 10, KD, with t(16q—;22q+).

type 46,XX,t(4q+;11q—). Since the proposita lacked the deleted metacentric C, she probably had inherited only the 4q+ and was thus trisomic for the distal third of the long arms of chromosome 11: 46,XX,4q+mat. Note the phenotypic difference from the patient found to be trisomic for the short arms of no. 11 (case 7).

Case 10: KD 071668

In regular karyotypes from the proposita and her father, one G-group chromosome is replaced by a medium-sized acrocentric, somewhat shorter than a D. Pronounced nonhomology of pair 16 in the father suggests the possibility of a translocation between a no. 16 and a G.

In four QM-stained metaphases from the father, both smaller intensely fluorescent

chromosomes 21 are present (fig. 10*b*). The long arms of one chromosome 22 are almost twice as long as those of its homolog because of distally transposed brightly fluorescent material. If the members of pair 16 are compared, the amount of intensely fluorescent material on the long arms of one of them appears to be reduced. The corresponding half of the intensity profile is clearly shortened, and the centromere index approaches 50% (fig. 8*d*). These findings are interpreted as a probably balanced translocation: 46,XY,t(16q−;22q+). Hence, the proposita most likely had trisomy for the distal third of the long arms of chromosome 16: 46,XX, 22q+pat.

Case 11: LR 082262

Initial chromosome studies on all three affected siblings were interpreted as normal. The mother's karyotype, however, showed an apparently balanced t(Dq+; F?−). A nonhomologous pair in the D group was subsequently detected in the three patients. In autoradiographic studies, one member of pair 13 was found to have elongated arms with an unusual absence of label at the distal end.

In four fluorescent karyotypes from the proposita, there appears to be some rather intensely fluorescent material added to the long arms of a no. 13 distal to the two characteristic bright bands. Pairs 19 and 20 are structurally normal (fig. 11*a*). Five QM-stained metaphases from the mother reveal an identical 13q+ and, in addition, a missing no. 20 replaced by a small acrocentric (fig. 11*b*). This chromosome appears similar to pair 22 in size and weak fluorescent pattern but can be distinguished easily by intensely fluorescent satellites on both no. 22s, which is an individually variable heritable feature [17]. This abnormal acrocentric thus represents a deleted chromosome 20. The missing brightly fluorescent part of the short arms appears to be transposed to the long arms of a no. 13. Therefore, the mother is probably a carrier of the balanced translocation 46,XX,t(13q+;20p−). Since the three children have normal F's in addition to the 13q+ inherited from the mother, they are trisomic for the bright region of the short arms of chromosome 20. The interpretation for the female sibling studied is expressed as 46,XX,13q+mat.

DISCUSSION

Progress in human cytogenetics has been dependent on the availability of techniques suitable for identifying specific chromosomes and recognizing their structural variations. Autoradiography with tritiated thymidine has permitted identification of more chromosomes than the standard technique, but the newly developed quinacrine mustard (QM) staining technique for chromosome analysis is superior to autoradiography for the following reasons: (1) all 24 different chromosomes can be identified; (2) the results are generally unequivocal; (3) it is less time consuming and less expensive, particularly since slides from the initial routine culture can be used; (4) a differential fluorescent labeling pattern within the chromosomes allows determination of points of chromosome breakage and rejoining and of the size of translocated or deleted chromosome parts. As with the standard technique, karyotyping of fluorescent metaphases can be done visually. But with photoelectric

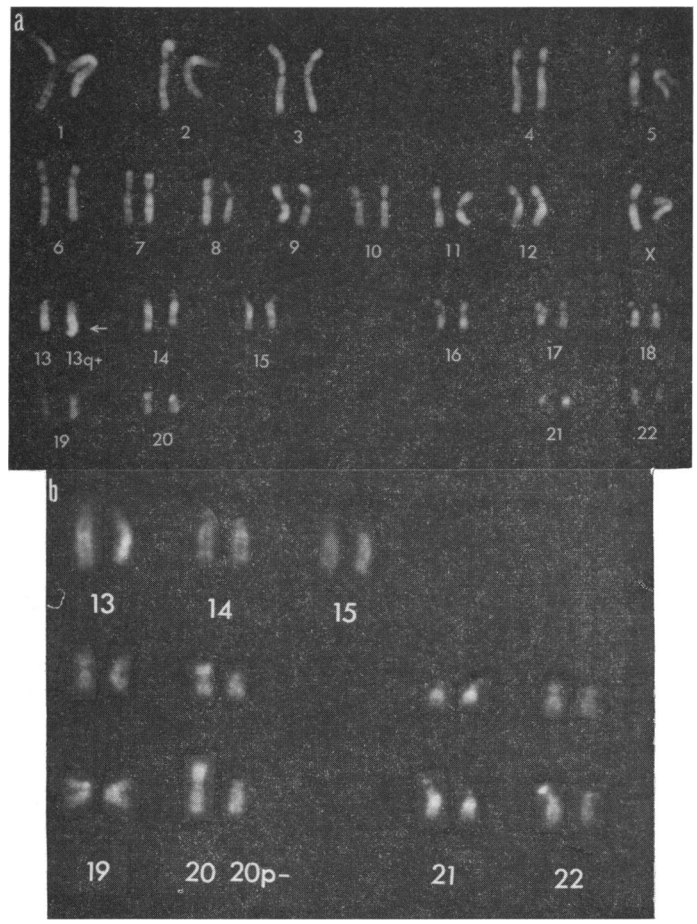

FIG. 11.—Case 11, LR: (*a*) Fluorescent karyotype of proposita; arrow indicates 13q+. (*b*) Partial fluorescent karyotype of LR's mother with t(13q+;20p—); D group and upper row of F and G groups are from one cell.

measurements of the fluorescent intensity distribution, as obtained with a microdensitometer, higher accuracy and objectivity is achieved, since the human eye is poorly equipped for observation of continuous transition in light intensity [4, 18]. Furthermore, in the study of structural chromosomal abnormalities, the profile curves are very useful for the location of breakpoints and for comparison of translocated fragments, as illustrated in figures 2, 6, and 8.

Using the QM fluorescent technique to study 16 individuals carrying translocations in a balanced or unbalanced complement, it was possible not only to identify but also to characterize specifically the abnormal chromosomes. This could be done because the fluorescent pattern is consistent in a given chromosome region, even after it has been translocated. For example, which set of arms in a metacentric chromosome is involved in a translocation can be determined by its fluorescent pattern (as in cases 1, 5, 10, and 11).

No evidence for truly reciprocal translocations was found among the 11 different rearrangements studied. This result was unanticipated, since translocations have been generally thought to be reciprocal. According to the telomere hypothesis, derived by Muller [21] from his work with *Drosophila* chromosomes, both ends of each chromosome have natural terminating elements (telomeres), and chromosomes need to have a telomere at each end in order to be stable and survive. Freshly broken ends are believed to be inviable because, lacking telomeres, they lead to end-to-end fusion of sister chromatids and ultimately to dicentric chromosomes which are not carried through mitosis. The existence of this breakage-fusion-bridge cycle was actually demonstrated by McClintock [22] in *Zea mays*. Therefore, at least two breaks are required for each surviving structural change. The hypothesis was challenged by the discovery of stable terminal deficiencies. Studying X-irradiation damage in Chinese hamster chromosomes, Hsu [23] demonstrated stabilization of the broken end by itself, when the break had been induced in a certain chromosome region which he consequently called an "interstitial telomere." Terminal deletions leading to partial deficiencies can therefore be explained as results of a single break in one of these areas. According to a similar concept by Uhl [24], the DNA within a chromosome is discontinuous, and the pieces are connected by "links" consisting mainly of protein. Breaks within such links or in bonds between the links and the DNA could lead to a viable state of the fragments.

Our findings, which raise anew the question of telomeres in human chromosomes and their possible relationship to translocations, are as follows: assuming that translocations are always reciprocal, the occurrence of a break in each of the affected chromosomes has to be postulated. In figure 12, the locations of these presumed breaking points in our patients are indicated. As shown, 50% of these breakpoints would have to be immediately subterminal. This is unexpected if the distribution of breaks within a chromosome is assumed to be random, as has been shown for breaks in human chromosomes induced by certain viruses and by radiation [25].

Subterminal breaks might be expected to produce minor changes in the fluorescent patterns of the affected chromosome secondary to loss of some chromosomal material. These could not be demonstrated in translocated chromosomes from our patients; for example, in the case of chromosome 4, the terminal bright band on the long arms appears to be fully retained after material has been translocated to it in case 9 (fig. 11*a*). The same is true for the weakly fluorescent long arms of chromosome 20, to which intensely fluorescent material has been added in case 5 (fig. 5*a*, *b*, *c*). But the possibility cannot be ruled out that subterminal breaks have occurred which produced fragments too small to be identified by this technique. However, an alternative explanation for our findings would be that translocations of human chromosomes can occur by a single break, with the fragment being transposed to the unbroken end of another chromosome and with the broken end of the deleted chromosome "healing" by itself. It would necessitate, however, assuming that unbroken chromosome tips are normally "sticky," so that fragments with freshly broken ends can be joined to form a new longer chromosome. No evidence of this has been reported previously. It is impossible to draw conclusions

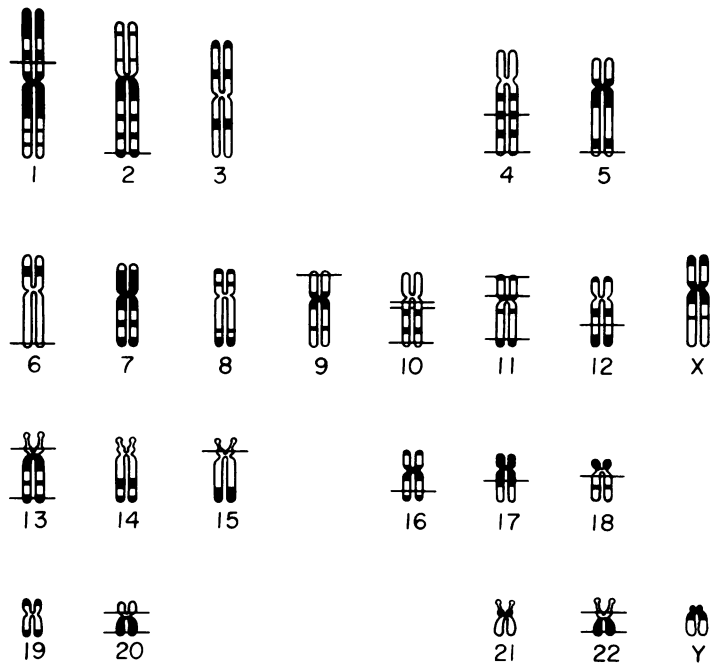

FIG. 12.—Schematic drawing of the most characteristic fluorescent band patterns on the chromosomes. Horizontal lines represent the location of breakpoints in cases 1–11, assuming the translocations are all reciprocal.

based upon such a small number of cases, especially since they represent a sample of translocations biased by ascertainment through routine chromosome analysis. Future fluorescent studies of chromosomal rearrangements in vivo and induced in vitro may be able to show whether the telomere hypothesis is valid for mammalian chromosomes. That true reciprocal translocations can be identified by the QM technique has been shown for the translocation *T(14;17)264Ca* in the mouse [26], in which chromosome pieces of almost equal size are exchanged.

After measuring the translocated fragments in cases 1–3, no apparent gain or loss of chromosomal material could be detected. It is not clear how the apparently balanced chromosome state in these patients is related to their abnormal phenotypes, particularly since balanced translocations are also detected in phenotypically normal individuals (e.g., one parent each of cases 5–11). Aside from the possibility of pure coincidence, position effect [27] may play a role; that is, the effect of a gene may be dependent upon its position with respect to neighboring genes. This phenomenon, well established in *Drosophila*, has not yet been demonstrated in humans. It might also be speculated that factors which caused chromosome breakage and subsequent de novo translocations in cases 1–3 had interfered with the normal development of the embryo by other mechanisms.

A potentially important achievement of QM staining lies in the definition of clinical entities associated with structural or numerical abnormalities involving

specific chromosomes within the C and F groups. This will permit more individualized genetic counseling, as illustrated by cases 2 and 6. Case 2 (L.S.), a carrier of t(10q—;22p+), can be counseled that about one-third of her offspring are at risk to be trisomic for two-thirds of the distal long arms of chromosome 10. Assuming that a specific chromosome abnormality produces certain phenotypic effects, the clinical findings in her future child having partial trisomy 10 may resemble the actual case 6 (K. W.), who was found to be trisomic for a slightly shorter piece of the long arms of no. 10. However, in our patients, certain congenital abnormalities, such as microcephaly, epicanthal folds, low-set ears, micrognathia, cleft palate, congenital heart disease, and developmental and growth retardation, are associated with partial trisomy for each of several different chromosomes. Therefore, the abnormal phenotype may be caused largely by the disturbed balance within the genetic complement rather than solely by the action of the specific genes which are present in three instead of two copies. This could interfere with any attempt to associate specific clinical findings with a certain chromosome change, although a complex syndrome, as in Down's syndrome with trisomy 21, might be correlated with a given chromosome aberration.

The Chicago nomenclature [17] is not adequate to convey all the information about translocations which can be obtained from fluorescent staining. First, the description of partially trisomic states in offspring of balanced translocation carriers is limited because the abbreviation symbols only permit description of the abnormal chromosome encountered in the proband and the designation of the parent from whom it is inherited (e.g., 2q+pat or 15p+mat). Since the derivation of the transposed chromosomal material can now be determined exactly, the nomenclature should be extended to include this knowledge. As a possibility, the number and arm of the chromosome from which the material was derived might be placed between the symbol for the arm to which it was translocated and the designation of the carrier parent: for example, 2q+(11p)pat for case 7 and 15p+(10q)mat for case 6. Parentheses have so far been used in connection with t for translocations and have enclosed the symbols for the chromosome parts involved. The above proposition would therefore not conflict with precedent. Second, if truly reciprocal translocations are found in humans, it is theoretically possible that chromosome fragments of equal size might be exchanged between two chromosomes. In this case, no elongated (p+ or q+) and no deleted (p— or q—) chromosome would be formed. As an appropriate designation for apparently balanced reciprocal translocations, arrows could be used in the following manner: t(20p⇄13q). When the existence of nonreciprocal translocations is established, a single arrow could be used: t(20p→13q).

SUMMARY

Staining with quinacrine mustard produces characteristic fluorescent band patterns on human metaphase chromosomes, permitting their specific identification. This technique has been applied to 16 individuals carrying various types of translocations in balanced or unbalanced states. The translocations t(1p—;13p+),

t(10q−;22p+), t(6q+;12q−), t(11p+;17q−), t(4q−;20q+), t(10q−;15p+), t(2q+;11p−), t(9p+;18q−), t(4q+;11q−), t(16q−;22q+), and t(13q+;20p−) have been identified and characterized. The amount of translocated material has been estimated from determination of the breakpoints by visual and photoelectric analysis of the fluorescent patterns. No evidence was found for the translocations being reciprocal. The question of reciprocal versus nonreciprocal translocations is discussed. The chromosome parts for which unbalanced individuals are trisomic have been determined. The value of the technique for the definition of new clinical entities associated with specific chromosomal abnormalities and its implication for more individualized genetic counseling is demonstrated. Suggestions are made for adjustment of the Chicago nomenclature to the results provided by the new technique.

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