SPECIFIC BANDING PATTERNS OF HUMAN CHROMOSOMES BY USE OF THE PROTEOLYTIC ENZYME TRYPSIN AND A BUFFERED GIEMSA STAIN

Thesis for the Degree of M. S.
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GARY L. MARSIGLIA
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ABSTRACT

SPECIFIC BANDING PATTERNS OF HUMAN CHROMOSOMES
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By

Gary L. Marsiglia

A modification of the Giemsa banding procedure of Seabright (1971), which employs the enzyme trypsin and a buffered Giemsa stain, was used in a systematic study of 10 controls and 14 patients known to have chromosomal rearrangements. The patients were selected from the residents at the Lapeer State Home and Training School and from among cases seen in the Genetics Counseling Clinic at Michigan State University.

Anomalies including sex chromosomal aberrations, autosomal deletions and both balanced and unbalanced autosomal translocations were found. A detailed discussion of the patients listing clinical findings, routine chromosomal analysis and an interpretation of the Giemsa bands with specific cytogenetic diagnoses are presented.

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Gary L. Marsiglia

A THESIS

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Michigan State University
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To Dotti

and Tim

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INTRODUCTION AND LITERATURE REVIEW

Recent advances in human chromosome methodology have made it possible to identify all 22 pairs of autosomes and the X and Y, by their characteristic bands. This present study employs one of the new techniques, Giemsa banding, using 10 controls and 14 patients with chromosomal anomalies. The patients were selected either to confirm a suspected diagnosis or to point out exactly which chromosomes, on the basis of the bands, were involved in complex structural rearrangements.

Early Methods

Lejeune, Gautier and Turpin in 1959, demonstrated that in Down's syndrome the individual possessed 47 instead of 46 chromosomes, being trisomic for a small acrocentric in the G group. This finding was the first aneuploid state described in man and added much impetus to the field of human cytogenetics. Shortly thereafter, two other trisomies involving small autosomes were described, trisomy D (Patau et al., 1960) and trisomy 18 (Edwards et al., 1960). Identification of other anomalies including translocations, deletions and sex chromosomal aberrations quickly followed.

At first the major difficulty encountered was that most of the chromosomes could not be individually distinguished from one another, except by group. A search for a method to distinguish pairs within the group was begun. Autoradiography was first applied to human chromosomes by Morishima et al., in 1962. Somewhat earlier, the basic features of chromosome organization and duplication had been revealed when Taylor et al. (1957) introduced tritiated thymidine into their study of Vicia faba chromosomes. Many of the first investigations on human chromosomes focused attention on the asynchronous pattern of DNA replication of the X chromosomes in females. They also suggested that some of the autosomes exhibited replication patterns that could be useful in characterizing chromosomes not distinguishable by morphology (Gilbert, et al., 1962). Modifications of techniques made it possible to autoradiographically identify the chromosomes of groups B, D and E (Schmid, 1963). Pairs 1, 2, 3 and 16 and frequently the Y were easily recognized by morphology and measurement. Chromosomes in groups F, G and C, except for the late replicating X, however, remained indistinguishable from one another.

Banding Patterns

In 1968, T. Caspersson, at the Institute for Medical Cell Research and Genetics, in Stockholm, Sweden, began experimenting with a fluorescent alkylating agent. It was

hypothesized that an alkylating agent might interact and accumulate in guanine-rich segments of DNA, specifically attacking the N-7 atom of guanine. A fluorescent alkylating agent whose presence on the chromosome could be detected by ultramicrofluorescence, would specifically allow one to visualize chromosomal loci with a high guanine content. Since both quinicrine and quinicrine mustard were highly fluorescent in the visible range, they were chosen to test this hypothesis. Both gave a rather diffuse fluorescence, but the mustard additionally demonstrated a clear pattern of cross striations which extended across both sister chromatids. By applying this stain to Vicia faba and Trillium, characteristic banding patterns for each chromosome were demonstrated (Caspersson et al., 1969).

The next step was to attempt this technique with human chromosomes. In 1970, Caspersson et al., showed that in man, each pair also produced distinct bands when stained with the quinicrine mustard. By this method each individual chromosome pair could be distinguished.

Originally, Caspersson had thought that the alkylating group in the quinicrine mustard was reacting with the DNA in two ways: (1) preferentially acting on the guanine moieties and (2) by intercalation in the double helix. He believed that this was why the bands appeared distinct for each chromosome pair. This was found not to be true when it was observed that quinicrine dihydrochloride, which lacks the

alkylating group and therefore should not bind to the guanine-rich areas, produced bands at the same sites as the mustard (O'Riordan et al., 1971). Proflavine and acriflavine, which are also fluorescent agents without alkylating groups, likewise produced the same bands.

Britten and Kohne in 1968, showed that a large fraction of the DNA of higher organisms reassociated faster than would be predicted from the DNA content of the cell. Another fraction of the DNA was observed to reassociate at the expected rate. These findings led them to conclude that certain areas of the DNA are redundant. Their survey further pointed out that the repeated DNA occurs widely in higher organisms and is ubiquitous among eucaryotes.

Hybridization of the DNA with radioactive nucleic acid, detectable by autoradiography, allowed an investigation of the distribution of the repeated sequences within the genome (Jones, 1970; Pardue and Gall, 1970). By using the technique of in situ hybridization these investigators showed that mouse satellite DNA, hybridized with the DNA in the centromeric regions of all the metaphase chromosomes except the Y. This was also observed for the RNA complementary to the satellite DNA. The centromeric regions were not only labeled, but also intensely stained with Giemsa. Because it is known that constitutive heterochromatin contains DNA that is primarily of the satellite type (Corneo et al., 1970), they concluded that the centromeric areas of the mouse were likewise

heterochromatic. [While many definitions of the term heterochromatin exist, whenever it is mentioned in this review, it will refer to the constitutive heterochromatin that contains most of the satellite DNA (i.e., the highly repetitive DNA), unless otherwise indicated.]

Arrighi and Hsu (1971) and Yunis, Roldan, Yasmineh and Lee (1971), working independently, demonstrated that the centromere regions in man could be selectively stained with Giemsa after denaturation followed by renaturation of the DNA in chromosome preparations. Since it had been shown that the centromeric regions in the mouse chromosomes are composed of repetitive DNA and since these regions are heavily stained by the Giemsa, it was suggested that the heavily "blocked areas" on human chromosomes also represented redundant DNA. The denaturation step consisted of NaOH treatment followed by the renaturation which was an incubation in saline sodium citrate (SSC). This renaturation procedure is similar to the renaturation properties of repetitive DNA. In this type of chromosome banding, the Giemsa is believed to stain all of the repetitive DNA sequences irrespective of their base composition whereas the quinicrine mustard is considered to bind repetitive DNA with a base composition specificity (Gagné et al., 1971).

By experimenting with modifications of this procedure, several investigators were able not only to achieve centromeric banding, but also banding in other parts of the

chromatids. The denaturation and renaturation steps varied, as did the clarity and numbers of bands. The procedures generally consisted of a NaOH denaturation followed by an incubation at 50-65°C for several hours (1-72) in a saline sodium citrate buffer or a potassium phosphate-sodium phosphate buffer (Hawkins, 1971; Schnedl, 1971; Ridler, 1971; Lomholt and Mohr, 1971; Drets and Shaw, 1971; Crossen, 1972). One technique, called the Giemsa 9 (Patil et al., 1971), obtained a differential staining by increasing the pH of the stain from 6.8 to 9. Another procedure, termed the ASG technique for acetic/saline/Giemsa (Sumner et al., 1971), fixed the slides in methanol and acetic acid, followed by an incubation for one hour at 60°C in 2xSSC, then stained in the Giemsa for 1.5 hours. This technique showed that the fixation itself denatures the DNA.

One procedure, however, reported by Dutrillaux and Lejeune (1971), displayed bands in the reverse order of the others and for this reason has been called the R band technique. Slides are placed in a pH 6.5 phosphate buffer at 87°C for 10 minutes, fast cooled to 70°C, rinsed in tap water and stained in a pH 6.7 Giemsa solution. Even though the method is quite similar to the others, the banding results are just the opposite.

While it was now possible to readily identify individual chromosome pairs by the position of their bands, an explanation of why the bands appeared as they do, remained a

matter of speculation. Drets and Shaw (1971) believed that the centric heterochromatin represented the rapidly annealing, highly repetitive DNA, while the bands scattered throughout the genome represented families of repeated sequences with fewer copies. The unstained interband regions could then be the sites of unique nucleotide sequences. Their hypothesis was further supported by the fact that a longer incubation period was necessary to reveal the bands. Schnedl (1971), believes that at least some of the bands might represent chromosome regions occupied by reiterated DNA, since it is known from the work of Britten and Kohne that the repetitive DNA renatures at a faster rate than other DNA. It should be pointed out, however, that the quinicrine fluorescence technique, does not involve any sort of renaturation process, yet produces bands very similar to those described by the Giemsa technique (Sumner et al., 1971). Thus, some factor other than repetition of DNA must be involved. A base-specific interaction would be a possibility, but there is no evidence this occurs.

A more recent and more reliable technique is one described by Marina Seabright (1971; 1972) of the Cytogenetics Unit in Salisbury, England. She explored the possibility that the banding was due to differential patterns of DNA-protein association along the length of the chromosome. To test this hypothesis, she employed the proteolytic enzyme, trypsin, and achieved distinct bands comparable to those

displayed by the other Giemsa techniques. The major advantage to this procedure, is its speed and consistency, since no incubation period is required and a greater proportion of the spreads are banded. Others, also using trypsin (Wang and Federoff, 1972), have postulated that the trypsin hydrolyzes the protein component of the nucleoproteins which have been denatured by the fixation procedures. This then allows the Giemsa to react with the exposed DNA, producing the bands.

The properties of Giemsa stain are undoubtedly important for band formation. Drets and Shaw (1971) have shown that acetic orcein did not produce banding, and Crossen (1972) obtained negative results with cresyl violet. Sumner et al., (1971), have reported that methylene blue gives only weak banding and it would appear that the combination of eosin and methylene blue is a factor in producing specific banding patterns.

Classification of the Bands

During the IVth International Congress of Human Genetics held in Paris in September, 1971, it was decided that because of the recent developments in human chromosome banding, that a conference on nomenclature be conducted in order to reconcile the many differences. The conference, sponsored by the National Foundation, will be published at a later date. The types of bands proposed by the committee

are as follows (Hsu, 1972):

1) The Q bands - fluorescent bands revealed by quinicrine mustard
2) The C bands - heavily stained regions revealed by a denaturation-renaturation process, usually centromeric
3) The G bands - a variety of techniques revealing cross bands using Giemsa stain
4) The R bands - the reverse bands of Dutrillaux and Lejeune, previously discussed.

MATERIALS AND METHODS

Chromosome cultures were prepared from peripheral leukocytes, using the macro-method. Blood was drawn by vena puncture into a syringe containing 0.1 cc. heparin, to prevent coagulation. This was then left in an upright position in order for the white cells to separate out from the red cells. After approximately 1 1/2 hours, 2 cc. of leukocyte enriched plasma was added to 8 cc. of Grand Island Biological Company Chromosome Media 1A. The cultures were incubated for 3 days at 37°C. 0.2 cc. of .004% colchicine was added to each culture to arrest the cells at metaphase. Incubation was then continued at 37° for an additional 3 hours.

The harvesting procedure was a modification of that of Moorhead et al. (1960). The cultures were removed from incubation and spun at 1600 RPM for 3 minutes in a centrifuge, leaving a button of cells. The supernatant was drawn down to 1 ml. followed by the addition of 5 ml. of warm (37°C) .075 M KCl to each culture for 8 minutes. Fixation of the cells consisted of 4 washes with Carnoy's solution (3:1 methanol: glacial acetic acid) for 10 minutes each. Eight to ten drops of the chromosome suspension were dropped from arm's length onto slides pre-chilled in 95% ethyl alcohol. The slides were flame dried in order to rupture the cells.

Many different Giemsa banding procedures were attempted and it was decided that a modification of the Seabright procedure (1971) gave the most consistent and most reliable results. Freshly harvested slides were placed in a 0.25% trypsin - GKN solution (see Appendix) for 15-25 seconds. GKN is a Ca^{++} and Mg^{++} free balanced salt solution. The slides were then rinsed thoroughly in two washes of 0.85% NaCl. At this point, it was possible to scan the slides under phase contrast microscopy to assess the action of the enzyme. If the spreads appeared slightly swollen, the slides were ready for the stain. If not, they were exposed to the trypsin for a longer period of time.

The stain was a 1:10 Fisher stock Giemsa (0.8 gm. powdered Giemsa, 50 ml. methanol, 50 ml. glycerol) to a 0.6 M Na_2HPO_4/KH_2PO_4 Sorensen buffer at pH 6.8 for 3 minutes. This was followed by a short rinse in the same pH 6.8 buffer. If the slides were understained, they could be returned to the Giemsa for a longer period of time and if they were overstained, they were returned to the buffer for a more thorough rinse.

The slides were scanned under bright-field on a standard Zeiss photoscope and photographed without a coverslip. Adox 35 mm. film was used and this was developed in D-19 (1:4) for 3 minutes at 68°C. Prints were made on Fotorite FPI #4 photographic paper.

RESULTS

Before any patient could be analyzed karyotypically by the Giemsa banding procedure, 10 controls were studied in order to establish a set of "normally" banded chromosomes for the survey. The banding patterns attained compared favorably with those of Seabright (1972), with the exception of the X, and the chromosomes were numbered according to her idiogram (Figure 1). Seabright's numbering corresponds to that used by Caspersson (1971) for the fluorescent studies, since these had already been well established at the Orly-Paris Conference on human chromosome nomenclature.

The following is a list of the individual chromosome pairs summarizing the banding patterns of each. The characteristics described are those which are most favorable for visual identification from photographic prints. The bands are discussed beginning with the distal end of the short arm and continuing to the distal end of the long arm.

Chromosome 1

This chromosome which is easily recognized by its morphology has distinctive banding patterns in each arm. The distal portion of the short arm appears unbanded followed by

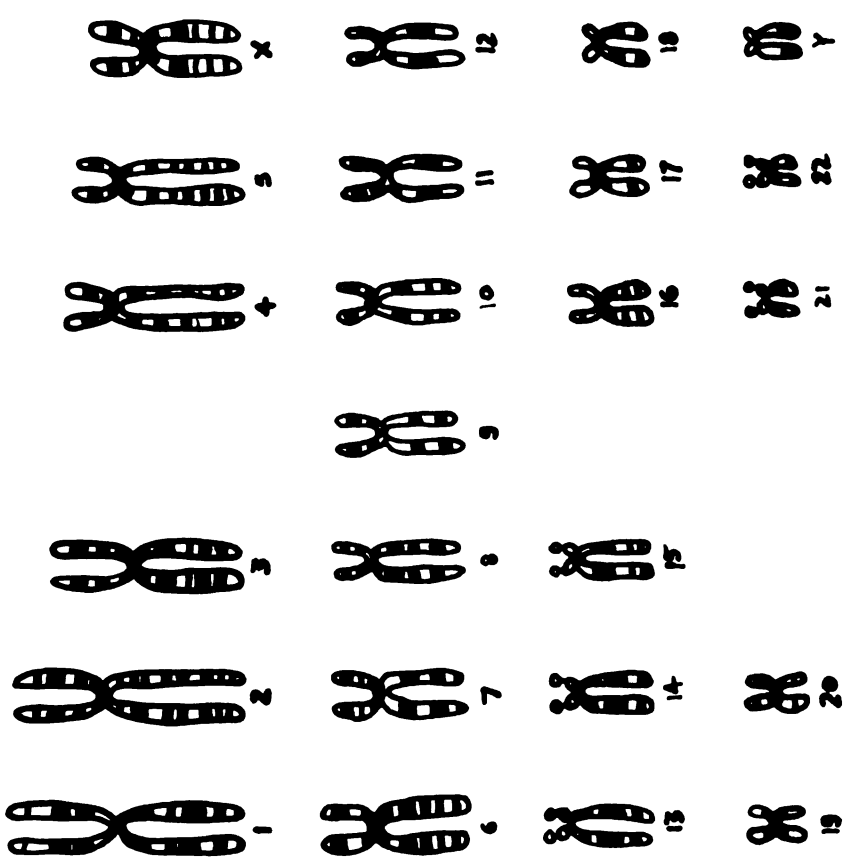

Figure 1. Seabright idiogram.

one faint band and two heavily stained bands nearer the centromere. The long arm is characterized by a dark staining band in the centromeric region, followed closely by a small dark band. This latter band is not easily distinguished from the centromeric band in most preparations and usually the two bands appear as one. These are followed by four bands throughout the rest of the length of the long arm, the second of which is the most prominent.

Chromosome 2

Both arms of this chromosome have fairly uniform banding. The short arm has 4 equally spaced bands, the second band which is in the middle, being the most intensely stained. The bands on the long arm are difficult to identify because they lie close together. According to Seabright there are 8 bands present, the two in the middle portion being the most intense. In this group of controls, the largest number of bands observed in the long arm was 6, the two nearer the centromere being more lightly stained than the remaining 4.

Chromosome 3

This chromosome has very distinct bands. Near the distal end of the short arm there is a strongly banded region. Also at the centromere and on either side of it, there

is a very distinct, wide, dark stained band. The distal end of the long arm has two darkly stained bands which may appear as one because of the intensity of the stain.

Chromosome 4

There are two bands on the short arm, one in the middle portion and one just above the centromere. The long arm has 5 bands, the first 3 which are prominent and separated from the distal pair by an unbanded region. The distal pair are very close together and may appear as one.

Chromosome 5

The short arm bands very similar to chromosome 4, displaying 2 bands, one very near the distal end and one at the centromere. The long arm has a distinct band just beneath the centromere, followed by a long heavily banded region in the middle portion. According to Seabright, this represents 4 dark bands very close together. There is also a prominent band at the distal end.

Chromosome 6

This chromosome is the largest member of the C group. The short arm has 2 distinct bands, one near the distal end and one at the centromere. These are separated by a clear unbanded region. The long arm also has a conspicuous band extending from the centromere. This is followed by two prominent bands and two pale distal bands.

Chromosome 7

The short arm displays a terminal dark band and a band near the centromere with a clear region between the two. The long arm has two very distinct bands on either side of the middle portion and a pale distal band.

Chromosome 8

This very submetacentric chromosome has two bands in the short arm, one distal and one above the centromere. The long arm has 4 bands, two very near the centromere and two more distally located.

Chromosome 9

The short arm has a dark terminal band and a band close to the centromere. The proximal portion of the long arm is unbanded followed by two darkly stained bands.

Chromosome 10

The short arm has 2 bands, one near the distal end and one at the centromere. There are 3 bands on the long arm, the first being the most heavily stained and found just below the centromere, the second in the middle of the long arm and the third at the distal end.

Chromosome 11

This chromosome displays two bands in the short arm, one near the distal end and one above the centromere. The long arm has a very broad, prominent band in the middle portion with unbanded regions on either side.

Chromosome 12

The pattern is very similar to chromosome 11, but the short arm has only one band. This is also the most submetacentric of the C group chromosomes. The long arm has a small band adjacent to the centromere and a median dark, broad band.

The X Chromosome

Visually this chromosome does not fit the idiogram of Seabright. There is a prominent band in the middle of the short arm. The centromere is usually unstained, but in some preparations staining has been observed. The long arm has 3 equally spaced bands, the first two being the most prominent.

Chromosome 13

None of the acrocentric chromosomes display distinct bands on the short arms. The long arm is best characterized by 3 heavy bands, two of which are near the distal end and because of the stain and close proximity, may appear as one.

Chromosome 14

The two darkly stained bands just below the centromere are characteristic for this chromosome. There is also an intense band at the distal end with a clear unbanded area between.

Chromosome 15

This chromosome has one broad band in the long arm somewhat near the centromere, with the distal portion appearing unbanded.

Chromosome 16

The short arm has one faint band near the distal end. The long arm has a characteristic intense band just beneath the centromere followed by two bands of lesser intensity.

Chromosome 17

The short arm is banded only at the centromere. The long arm is also banded at the centromere and has a band near the distal end.

Chromosome 18

The short arm appears unbanded. Like chromosome 17, the long arm has 2 bands, but the centromere is

non-staining. There is one prominent band below the centromere and one at the distal end.

Chromosome 19

This chromosome bands only at the centromere with the remainder of the chromosome appearing very pale.

Chromosome 20

There is a faint band in the medial portion of the short arm, a banded centromere, and a faint band in the medial portion of the long arm.

Chromosome 21

The long arm of this chromosome is almost entirely banded, with only the most distal portion unbanded.

Chromosome 22

This chromosome is readily identified by its darkly stained centromeric region. Thus, there is no difficulty in distinguishing chromosomes 21 and 22.

The Y Chromosome

Besides being recognized morphologically, this chromosome has a fairly heavy band on the distal half of the long arm.

A representative karyotype of one of the controls, displaying the bands as they would appear in an apparently normal individual, is shown in Figure 2.

Use of the Banding Technique in Cytogenetic Diagnosis

Since the Giemsa banding procedure utilizing trypsin, has made it possible to distinguish individual chromosome pairs and to recognize their structural variations, this technique was applied to 14 patients displaying translocations, deletions and trisomies. In each case it was possible to specifically identify the abnormal chromosomes involved.

A summary of the patients, together with the results from routine staining and Giemsa banding is given in Table 1. Specific information including clinical findings and interpretation of the Giemsa bands will be analyzed for each unique case separately.

Case #1: K. H. born 11-12-57 (See Figure 3)

A mildly retarded girl, with growth retardation and microcephalus was referred for a possible chromosomal abnormality. Buccal smear revealed 37% with two sex chromatin bodies and karyotype analysis showed a 47,XXX. Autoradiography was considered but not performed.

Giemsa banding confirmed the diagnosis demonstrating the extra C group chromosome to be an X.

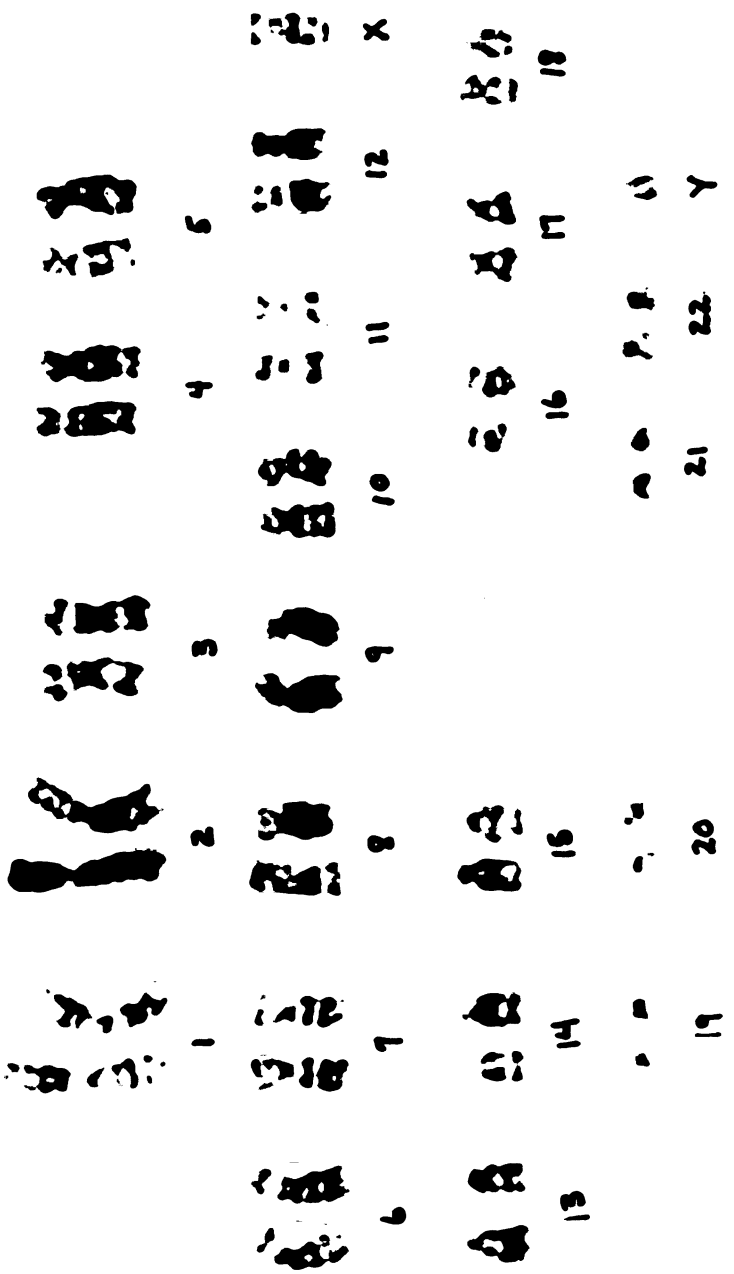

Figure 2. Normal male karyotype, 46,XY.

Table 1

Summary of patients and results by routine karyotype analysis and Giemsa banding

Patient	Routine analysis	Giemsa banding
Case #1 K. H. born 11-12-57	Extra C group chromsome believed to be an X. Autoradiography considered but not done. 47,XXX	47,XXX
Case #2 J. G. born 11-27-51	47,XYY	47,XYY
Case #3 C. K. born 7-5-22	Believed to be a Klinefelter's mosaic, 47,XXY/48,XXYY	48,XXYY
Case #4 K. P. born 5-11-60	Metacentric chromosome closely resembling a #3, thought to be an X isochromosome. 46,X iso $\bar{x}$	46, X iso $\bar{x}$
Case #5 N. C. born 12-24-71	Deletion of the short arm of a B group chromosome thought to be #5 because of phenotype of Cri-du-Chat. 46,XY,Bp-	46,XY,5p-

Table 1. Continued.

Patient	Routine analysis	Giemsa banding
Case #6 N. F. born 4-11-42	Deletion of the short arm of an E group chromosome 46,XX,Ep-	46,XX,18p-
Case #7 M. P. born 10-23-56	46,XX,G-,t(GqGq)+	46.XX,21-,t(21q21q)+
Case #8 C. C. born 6-10-49	46,XY,G-,t(GqGq)+	46,XY,21-,t(21q21q)+
Case #9 W. C. born 6-19-52 (sib of Case #8)	46,XY,G-,t(GqGq)+	46,XY,21-,t(21q21q)+
Case #10 C. L. born 5-19-33	46,XY,D-,t(DqGq)+	46,XY,14-t(14q21q)+
Case #11 M. H. born 1944 (mother of Cases #12 and #13)	Balanced translocation (carrier) between a G and distal end of long arm of a D. 46,XX,Dq-,G-,t(DqGq)+	46,XX,13q-,21-, t(13q21q)+

Table 1. Continued.

Patient	Routine analysis	Giemsa banding
Case #12 A. H. born 12-22-68 (sib of Case #13)	Same results as Case #12 46,XX,Dq-,G-,t(DqGq)+	46,XX,13q-,21-, t(13q21q)+
Case #13 P. H. born 7-30-66	Revealed an extra acrocentric smaller than a D and larger than a G. 47,XY,t(DqGq)+	47,XY,t(13q21q)+
Case #14 J. K. born 8-69	Complex chromosomal rearrangement involving a chromosome of A1, B and C groups. Assumed to be a double translocation. 46,XY,t(1q+,Bp+,Cp-q-)	46,XY,t(1q+,5p+,8p-q-) Probably a balanced translocation since there is no apparent loss of chromosomal material.

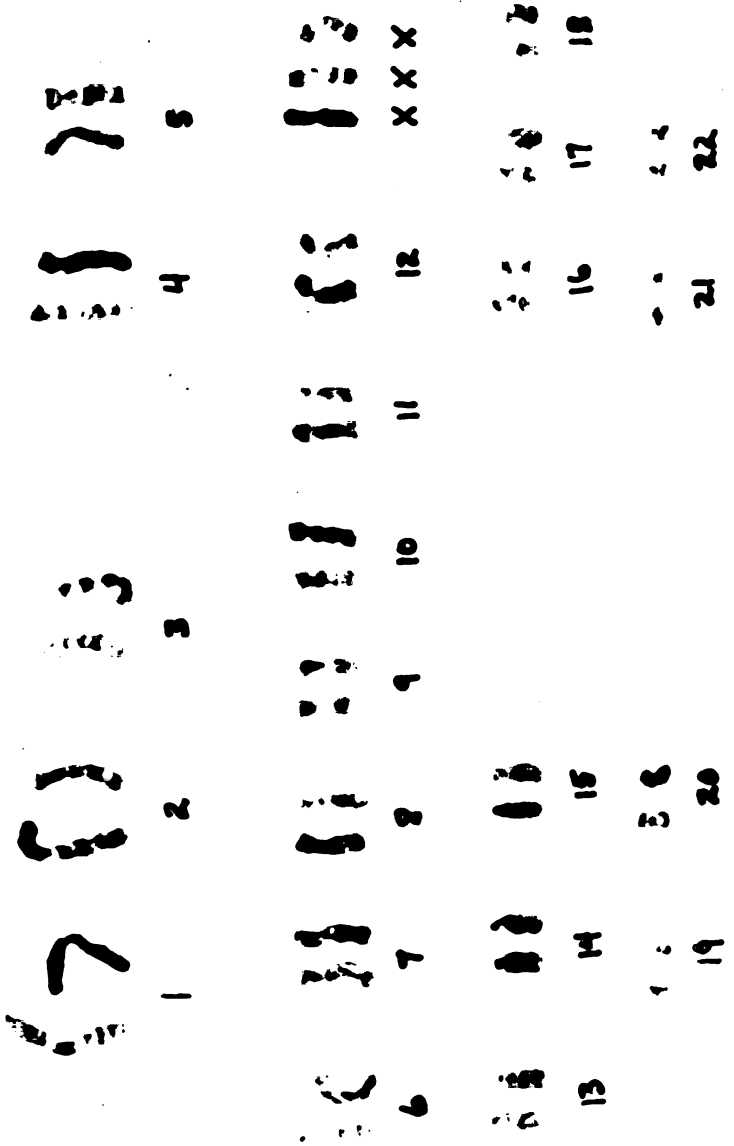

Figure 3. Case no. 1, 47,XXX, complete karyotype.

Case #2: J. G. born 11-27-51 (See Figure 4)

This patient was initially referred for chromosomal analysis as a possible XYY. His clinical findings included tall stature, thrombophlebitis, acne and an essential tremor. These are all consistent with the diagnosis of XYY. The patient displayed no tendency towards aggressive behavior, but did experience occasional, uncontrollable outbursts of temper. He was also chronically depressed with suicidal tendencies. His intelligence was estimated to be low average although no psychometric testing was performed.

Routine chromosomal analysis revealed a modal count of 47 with 6 chromosomes in the G group, the extra one considered to be a Y. Giemsa banding confirmed this diagnosis, showing two Y chromosomes, both heavily banded on the distal half of the long arm.

Case #3: C. K. born 7-5-22 (See Figure 5)

Standard karyotype analysis on C. K. displayed a possible mosaic Klinefelter's, 47,XXY/48,XXYY. The patient exhibited no gynecomastia and had normal external genitalia. He does have an essential tremor and varicose veins. He is the ninth of 11 children, has an IQ of 41 and a history of repeated escapes from the state home in which he is institutionalized. Ill-tempered at times, he is destructive and difficult to control.

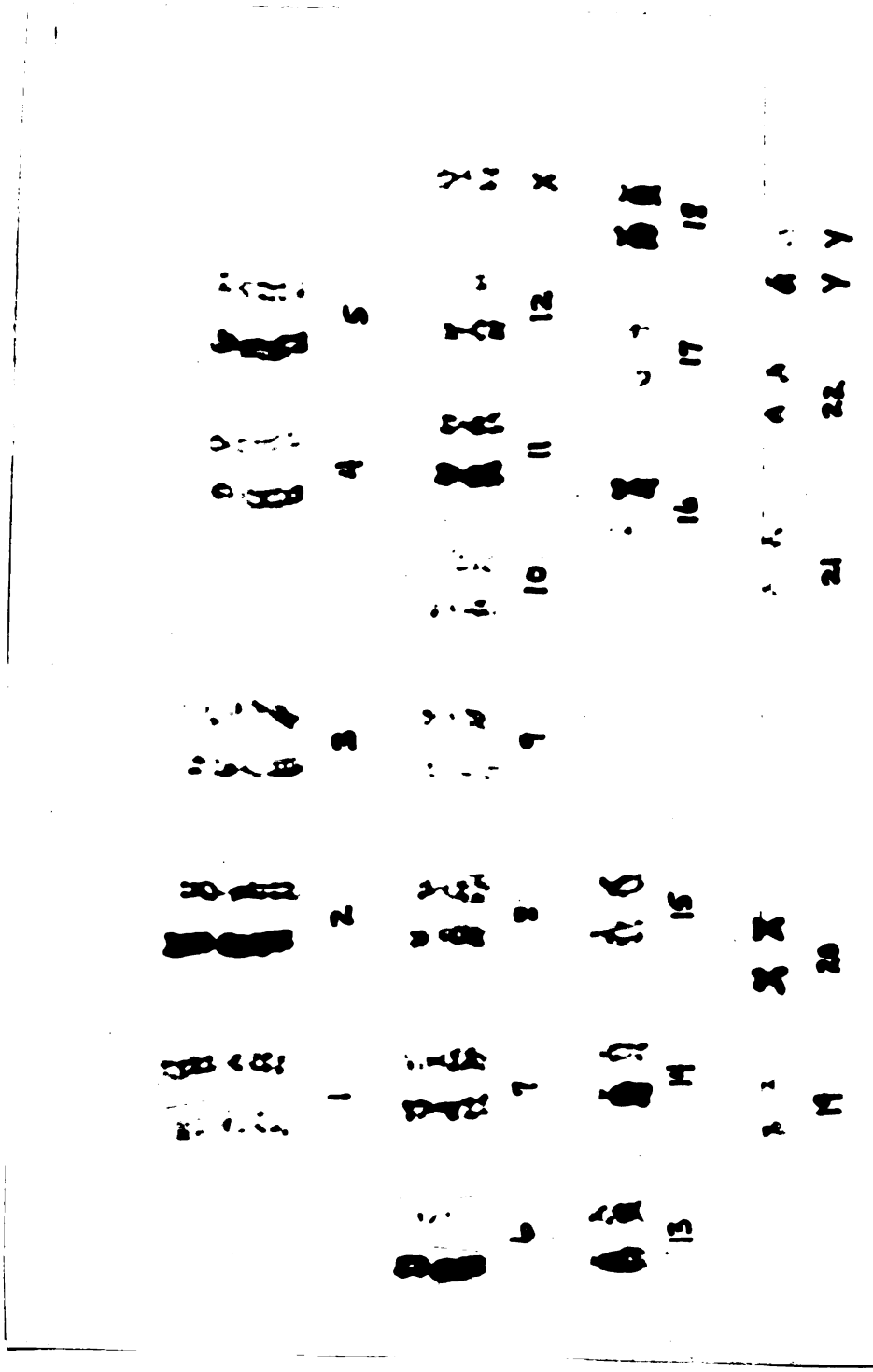

Figure 4. Case no. 2, 47,XYY, complete karyotype.

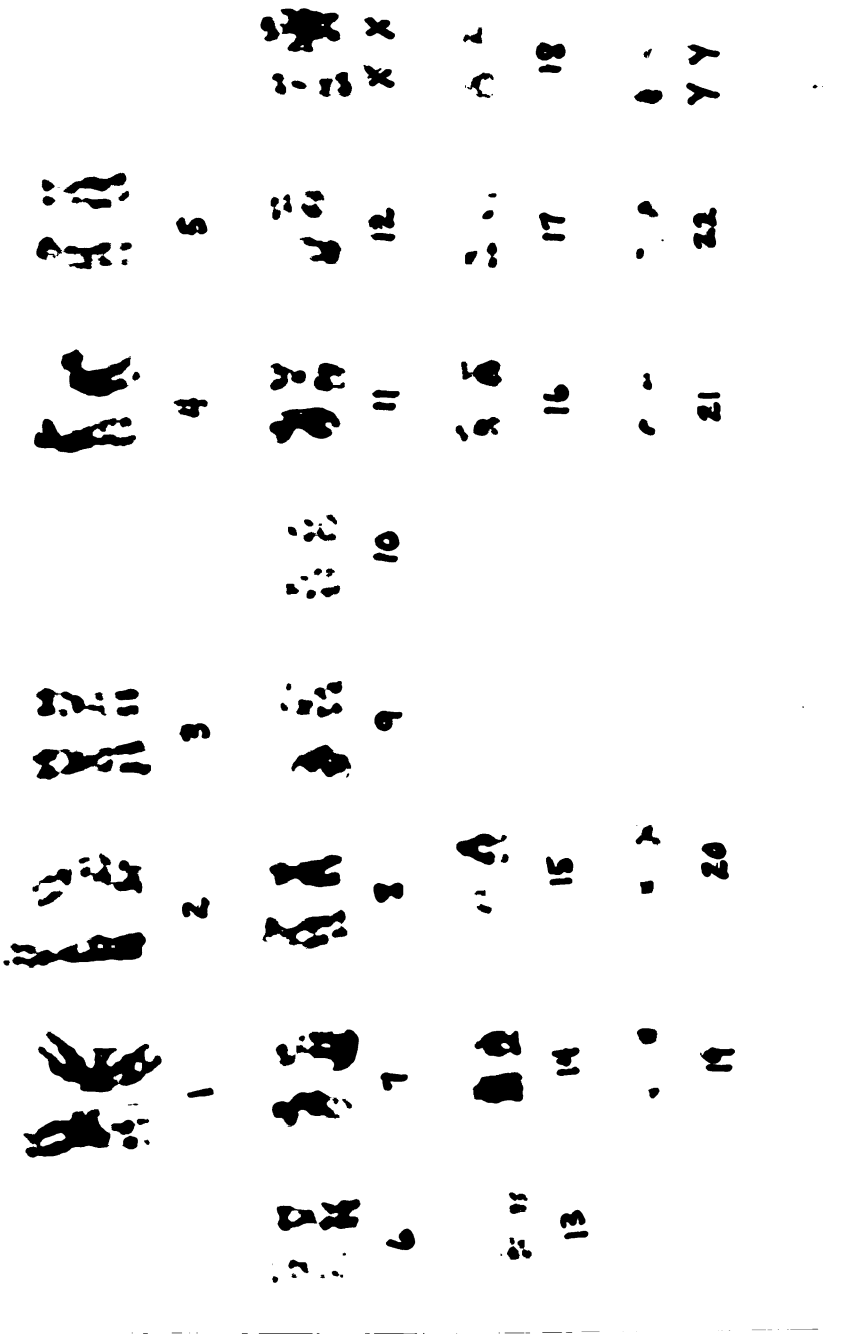

Figure 5. Case no. 3, 48,XXYY, complete karyotype.

Giemsa banding revealed a modal count of 48 with no evidence for mosaicism, 48,XXYY. The extra C group chromosome was identified as an X displaying one prominent band in the short arm, and 2 bands in the long arm. In this particular case, the centromere was also banded. The extra G group chromosome by morphology and distinctive banding on the distal region of the long arm was identified as a Y.

Case #4: K. P. born 5-11-60 (See Figures 6a and 6b)

This 12 year old female was referred for chromosome studies because of extreme short stature. She also displayed severe scoliosis, a cafe-au-lait spot, perceptual problems and a wide carrying angle. Buccal smear results were not consistent with those of a normal female, therefore chromosomal analysis was done to entirely rule out the possibility of Turner's syndrome.

Routine staining revealed a modal count of 46 with one of the C group chromosomes being completely metacentric, resembling an A3 by size. It was suggested that this was an isochromosome for the long arm of one of her X chromosomes. Thus, K. P. was trisomy for the long arm of the X and monosomy for the short arm, 46,X iso $\overline{x}$.

Giemsa banding exhibited one normally banded X in the C group, and one metacentric chromosome banded identically in both arms, resembling the pattern in the long arm of the normal X chromosome. It was easily identified from the #3 since

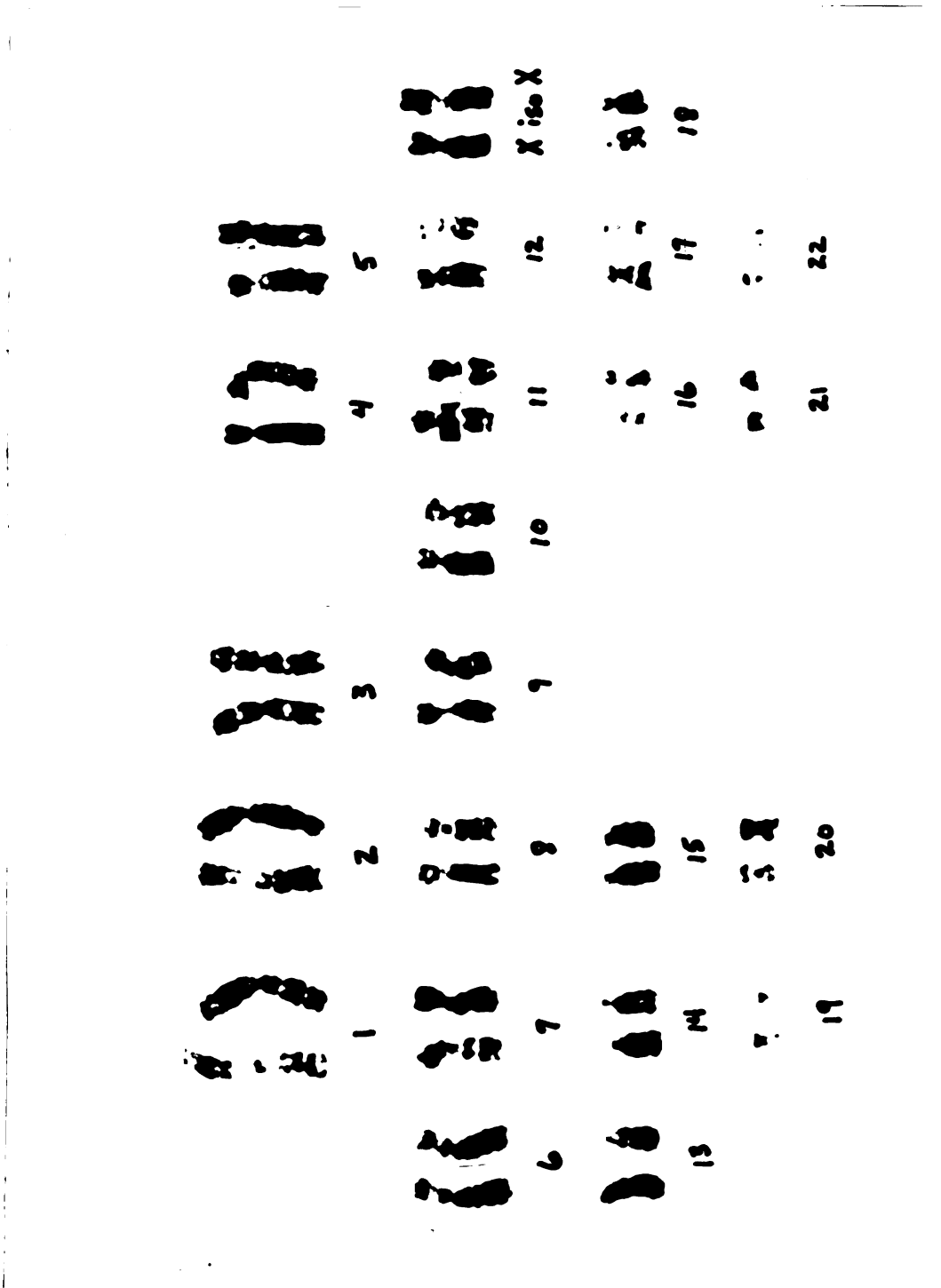

Figure 6a. Case no. 4, 46,X iso x, complete karyotype.

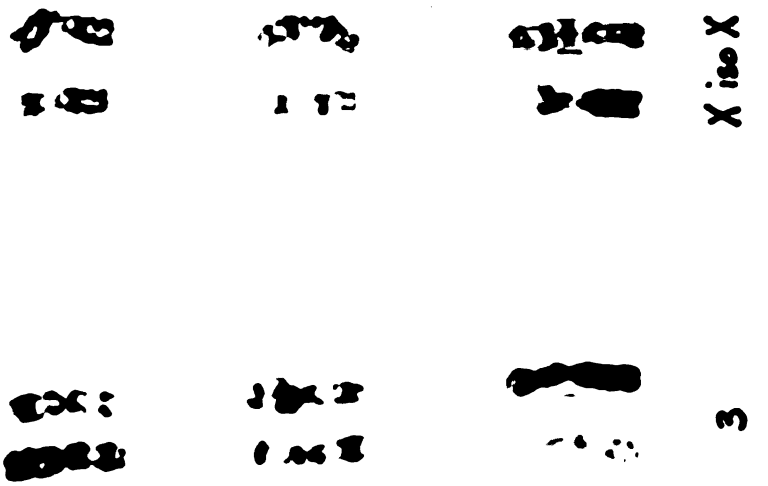

Figure 6b. Case no. 4, partial karyotype.

the 3 has a heavily banded centromere and the iso X has an unbanded centromere. The banding patterns reinforced the routine analysis diagnosis.

Case #5: N. C. born 12-24-71 (See Figure 7)

This very odd looking child was noticed in the new born nursery to have a high-pitched, whining and weak cry. Physical examination revealed an inactive white male, small in size with microcephaly, micrognathia, epicanthal folds, hypotonia, bilateral simian creases, pale mottled complexion and possible mental retardation. Pulmonary problems characterized by wheezing, congestion and "bubbly" breath sounds were also noticed.

Karyotypic analysis was requested because of the odd cry and physical findings. It revealed an apparent deletion of the short arm of one member of the B group chromosomes, 46,XY,Bp-. Because of the phenotypic characteristics of Cri-du-Chat, the deletion was assumed to be a no. 5.

Giemsa banding procedures revealed 3 normal B's, two of which show the pattern characteristic of a no. 4 and one which shows that of a no. 5. An extra deleted chromosome has a Giemsa pattern consistent with the long arm of a no. 5 showing a band just beneath the centromere followed by a long heavily banded region in the middle portion. Almost all of the entire short arm is missing.

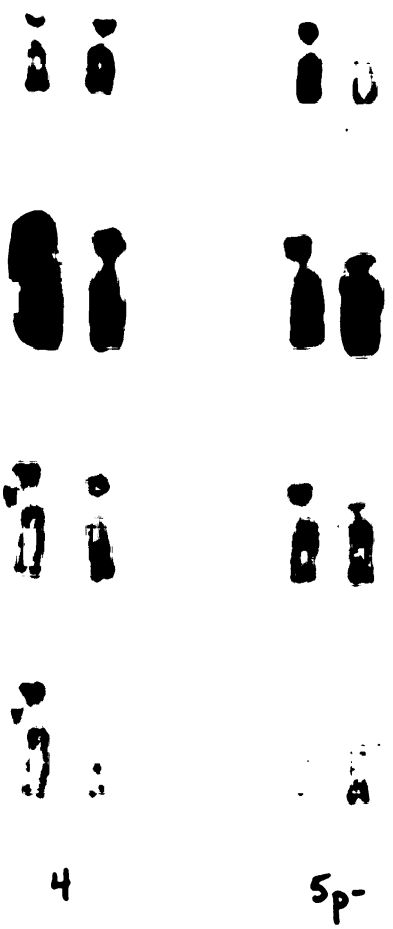

Figure 7. Case no. 5, 46,XY,5p-, partial karyotype.

Case #6: N. F. born 4-11-42 (See Figures 8a and 8b)

This individual, a resident at the Lapeer State Home and Training School, was initially referred for chromosomal analysis because she was thought to exhibit some of the Mongoloid characteristics. There were no outstanding physical findings aside from moderate retardation.

Chromosome studies displayed a 46 count with a deletion of the short arm of an E group chromosome, 46,XX,Ep-. Giemsa banding revealed a deletion of the entire short arm of a no. 18, the long arm consistently showing 2 distinct bands, one beneath the centromere and one at the distal end, 46,XX,18p-.

Case #7: M. P. born 10-23-56 (See Figures 9a and 9b)

Case #8: C. C. born 6-10-49 (See Figures 10a and 10b)

Case #9: W. C. born 6-19-52 (See Figures 11a and 11b)

Cases 7 through 9 were all diagnosed as Down's syndrome at birth. All three clinically present the classical features of Down's syndrome including epicanthal folds, slightly protruding tongue, simple ears, spade hands, simian crease, short stubby incurving little finger and hyperextensibility of all joints.

Routine chromosomal analysis in each case produced a modal count of 46. Karyotypes showed only 3 chromosomes in group G (excluding the Y) and an extra metacentric

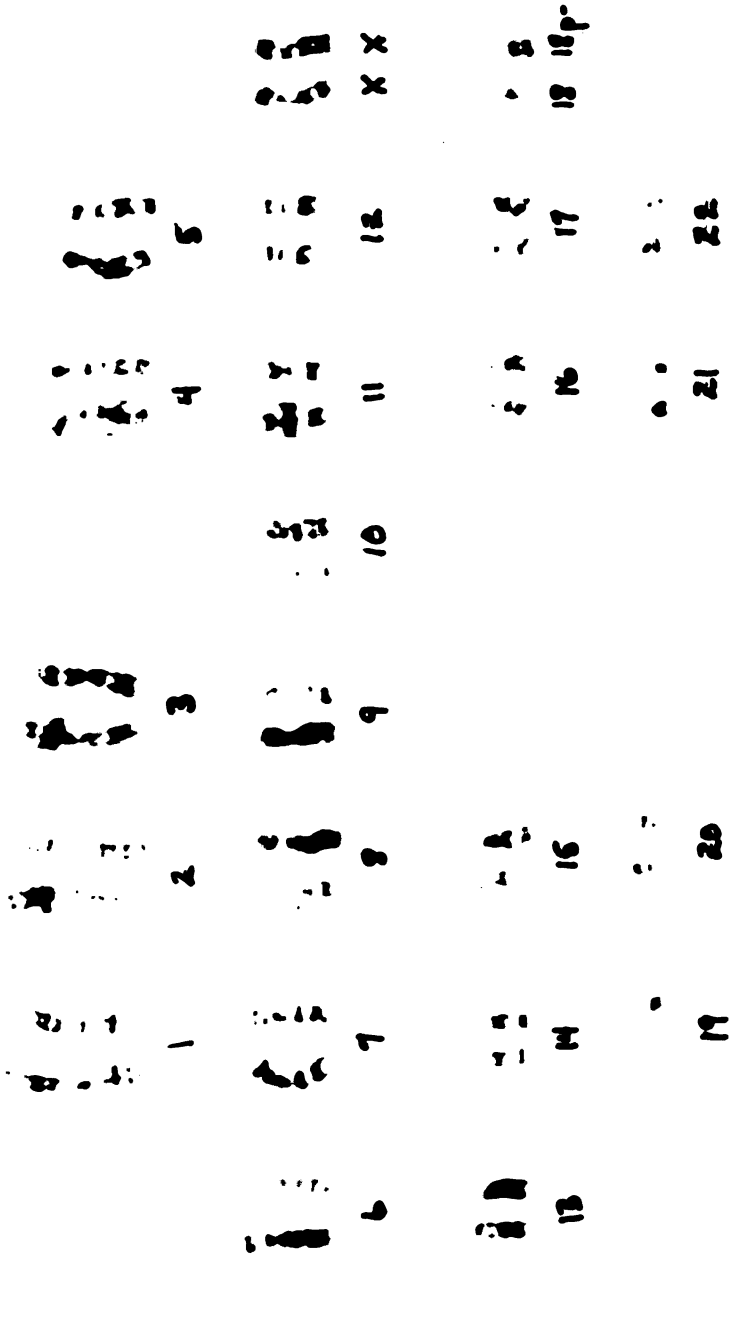

Figure 8a. Case no. 6, 46,XX,18p-, complete karyotype.

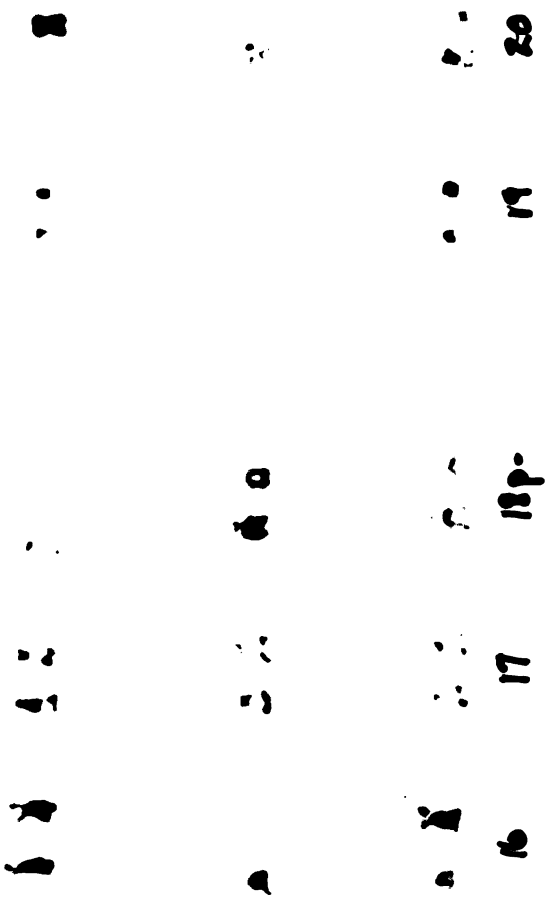

Figure 8b. Case no. 6, partial karyotype.

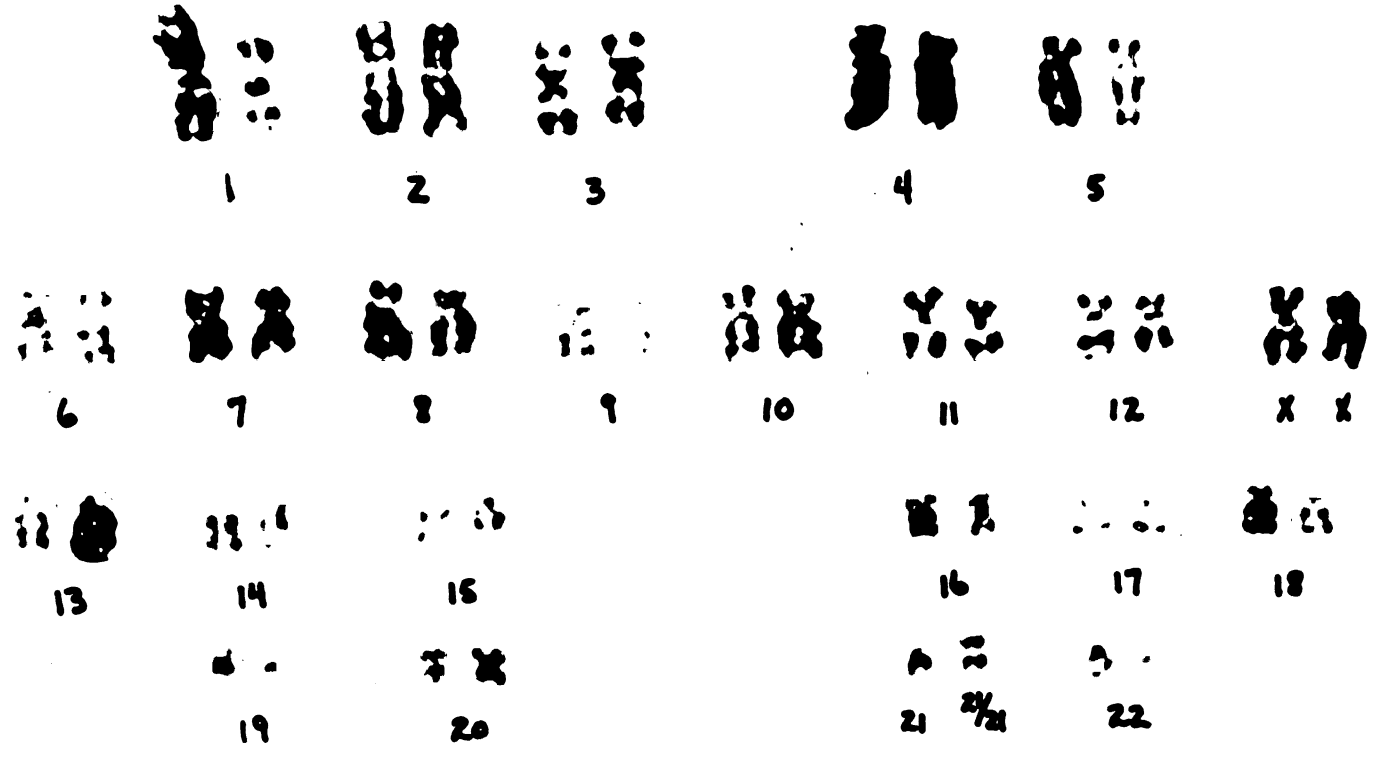

Figure 9a. Case no. 7, 46XX,21-,t(21q21q)+, complete karyotype.

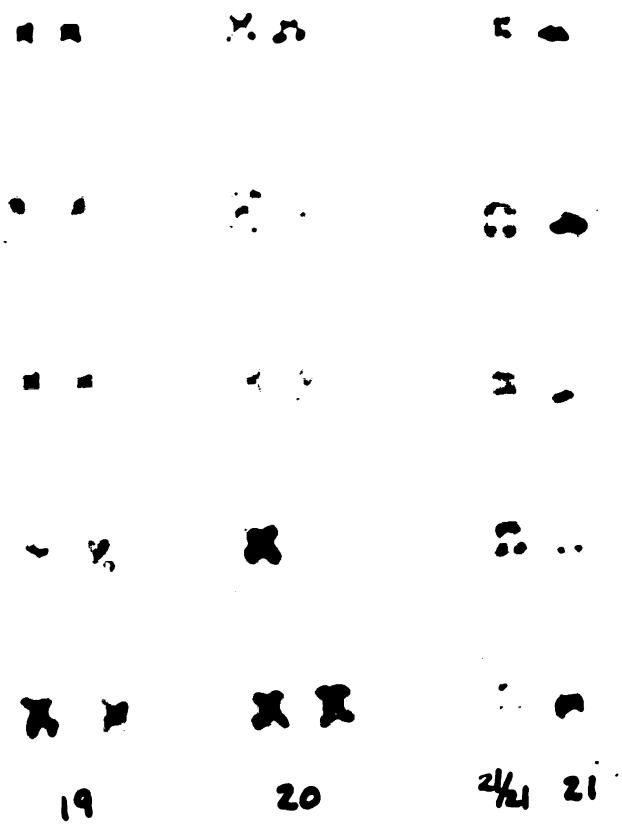

Figure 9b. Case no. 7, partial karyotype.

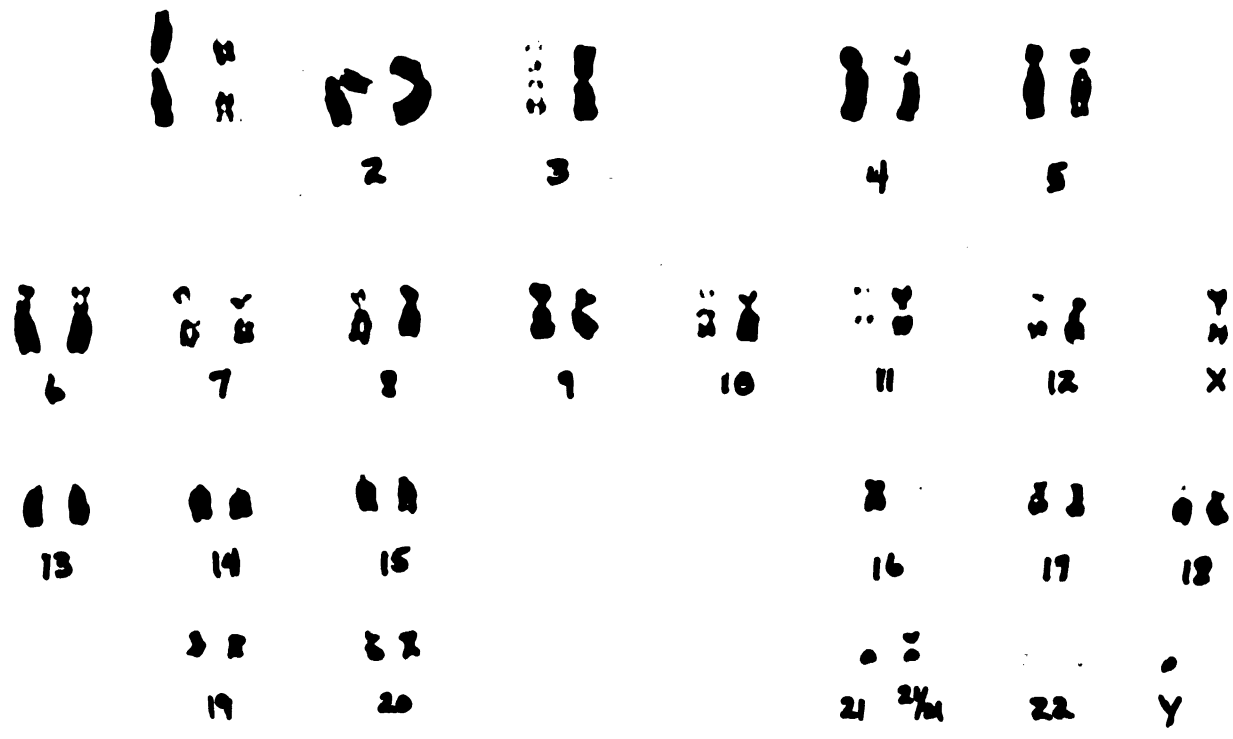

Figure 10a. Case no. 8, 46,XY,21-,t(21q21q)+, complete karyotype.

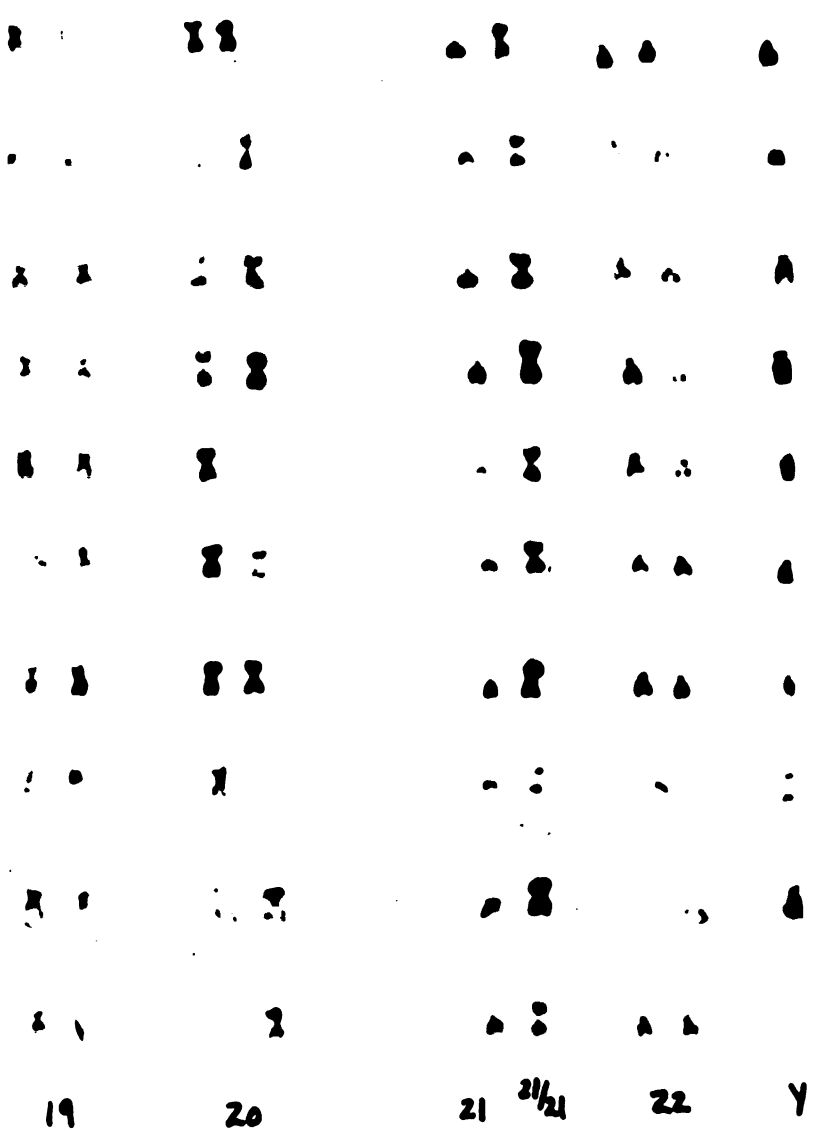

Figure 10b. Case no. 8, partial karyotype.

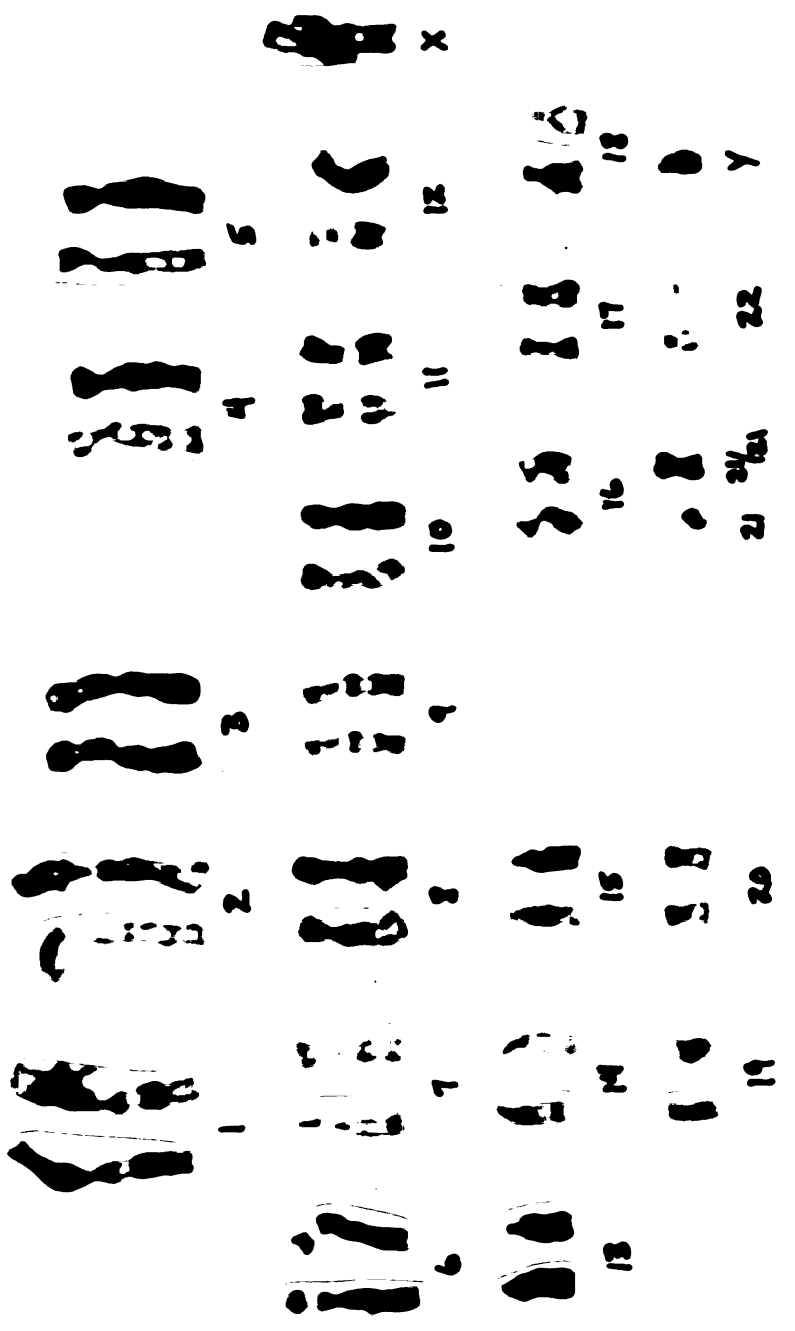

Figure 11a. Case no. 9, 46,XY,21-,t(21q21q)+, complete karyotype.

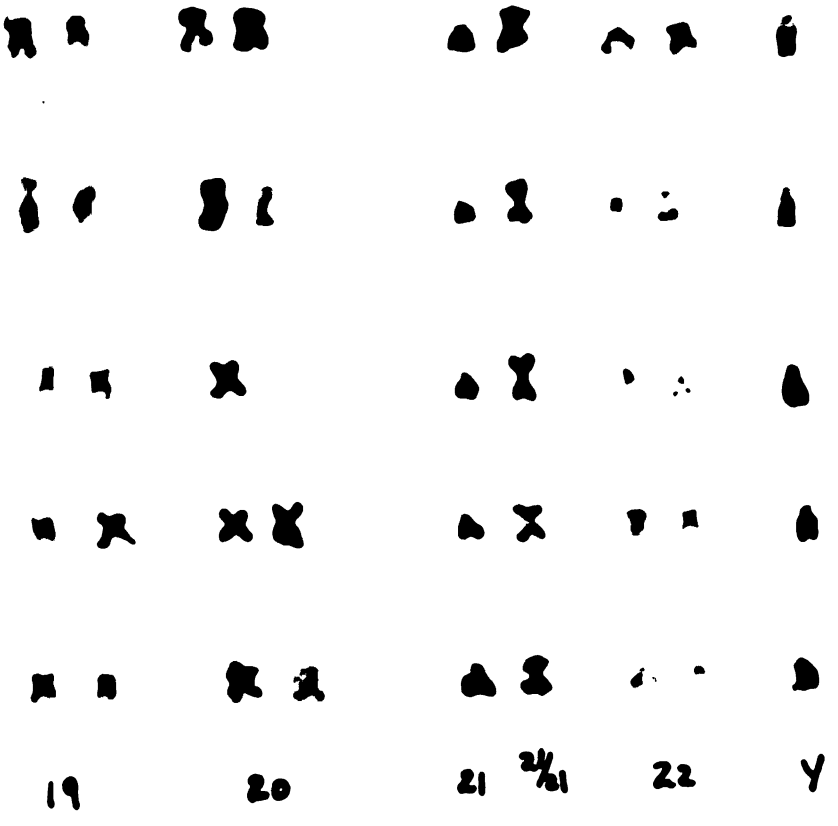

Figure 11b. Case no. 9, partial karyotype.

chromosome resembling members of the F group chromosomes. Morphologically this abnormal G group chromosome can be the result of a 21/21 translocation, a 21/22 translocation or an isochromosome for the long arm of a no. 21.

M. P. was the youngest of four children. Her mother and father were both shown to be karyotypically normal, indicating a sporadic translocation in the patient, 46,XX,G-, t(GqGq)+.

C. C. and W. C. are siblings and the only children in the family. There is no report of miscarriages or stillbirths. Their mother is karyotypically normal, but their father has a 45 count and is a G/G translocation carrier. Because both children are affected, it was assumed that the father of C. C. and W. C. carried a 21/21 translocation or an isochromosome for the long arm of a no. 21. In either case, he could produce only Down's children. Both boys were given a cytogenetic diagnosis of 46,XY,G-,t(GqGq)+.

Giemsa banding in all 3 cases revealed a 21/21 translocation. The small metacentric resembling by size an F, was completely heavily banded. Thus, it could be easily differentiated from the F group chromosomes which display very little banding.

Case #10: C. L. born 5-19-33 (See Figure 12)

This patient was referred for chromosomal analysis because of his characteristic mongoloid features. He also

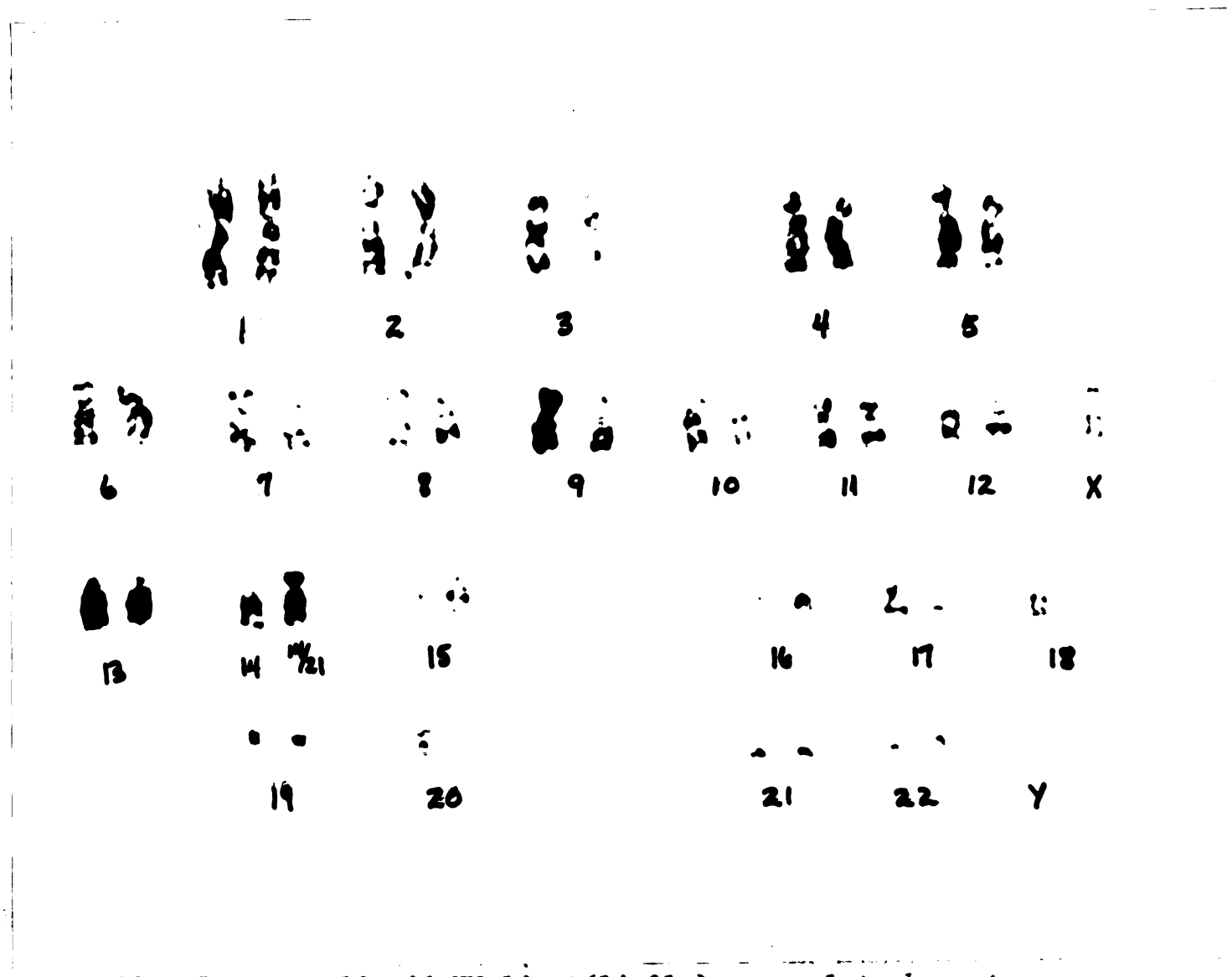

Figure 12. Case no. 10, 46,XY,14-,t(14q21q)+, complete karyotype.

had a speech problem and a behavioral disorder. This latter is quite unusual for Down's children who are considered to be good natured individuals.

Routine staining revealed a count of 46 with 5 normal D group chromosomes and an extra submetacentric chromosome resembling in size a C group chromosome. This additional chromosome was thought to have originated through a translocation between a supernumerary no. 21 and a member of the D 13-15 group, 46,XY,D-,t(DqGq)+.

Giemsa banding confirmed a D/G centric fusion and in addition specifically identified the chromosomes in question as the long arms of a no. 21 and a no. 14, 46,XY,14-,t (14q21q)+. The short arm of this submetacentric was actually the long arm of a no. 21, being nearly completely banded. The long arm of this chromosome was the long arm of a no. 14 displaying two dark bands just below the centromere and one dark band at the distal end.

Case #11: M. H. born 1944 (See Figures 13a and 13b)

Case #12: A. H. born 12-22-68 (See Figure 13b)

Case #13: P. H. born 7-30-66 (See Figures 14a and 14b)

Originally P. H. was clinically suspected to have Cornelia de Lange syndrome. At birth he had cleft lip and palate and bilateral polydactly on his hands and feet. When he was seen for chromosomal analysis his clinical manifestations

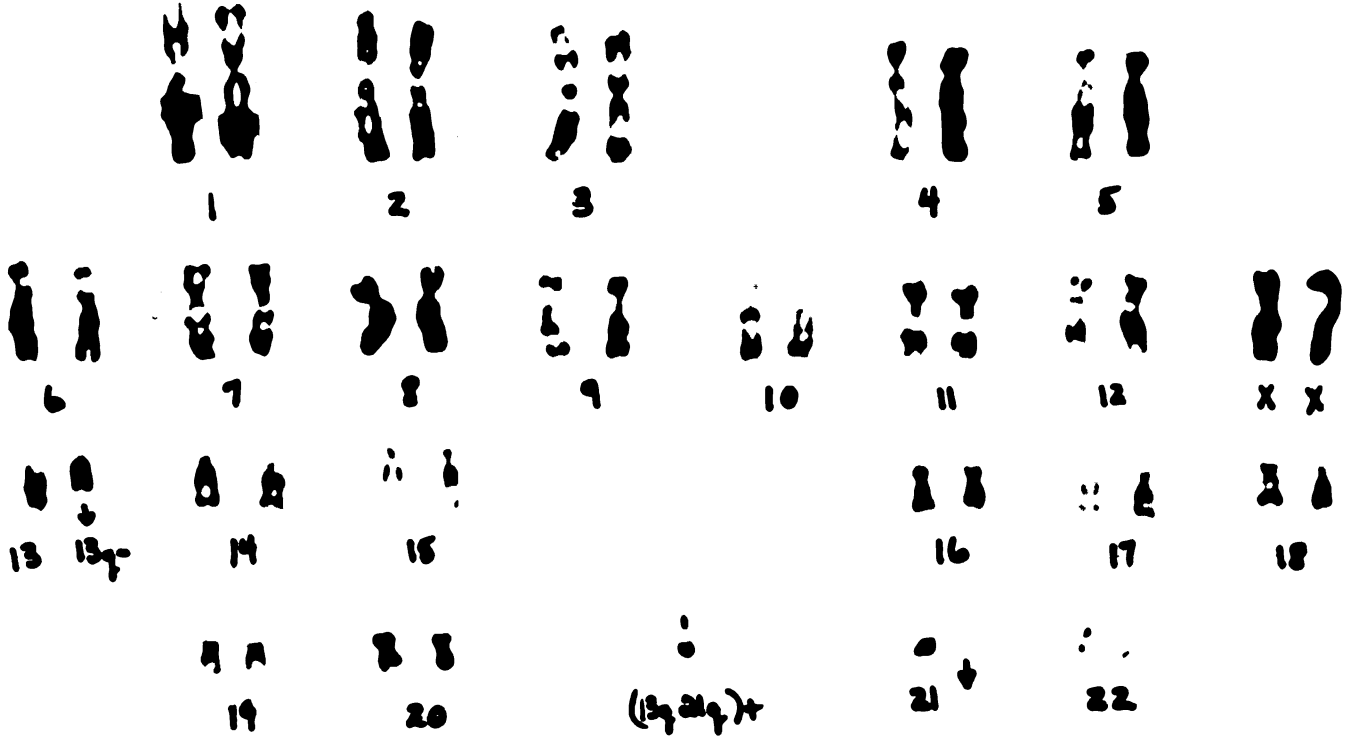

Figure 13a. Case no. 11, 46,XX,13q-,21-,t(13q21q)+, complete karyotype.

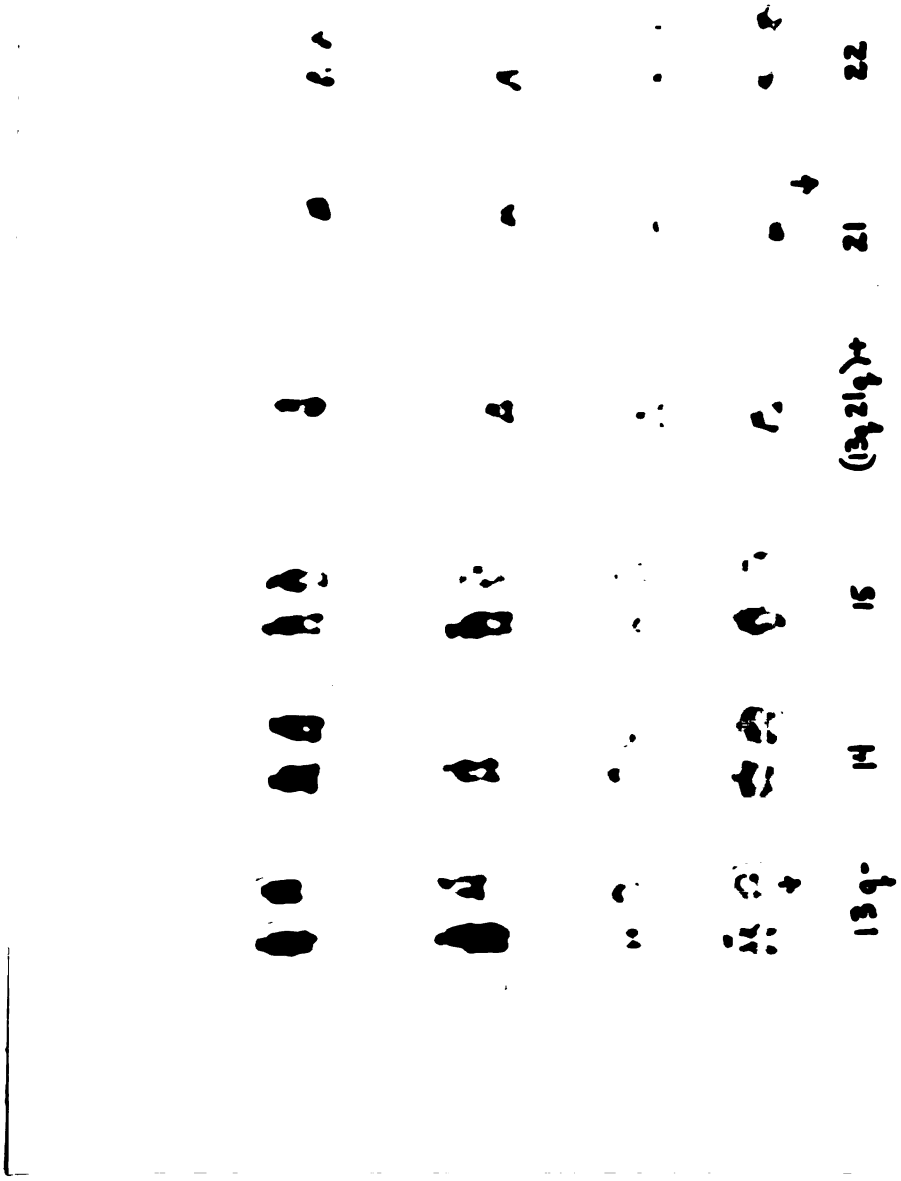

Figure 13b. Cases no. 11 and 12, partial karyotype.

Figure 14a. Case no. 13, 47,XY,t(13q21q)+, complete karyotype.

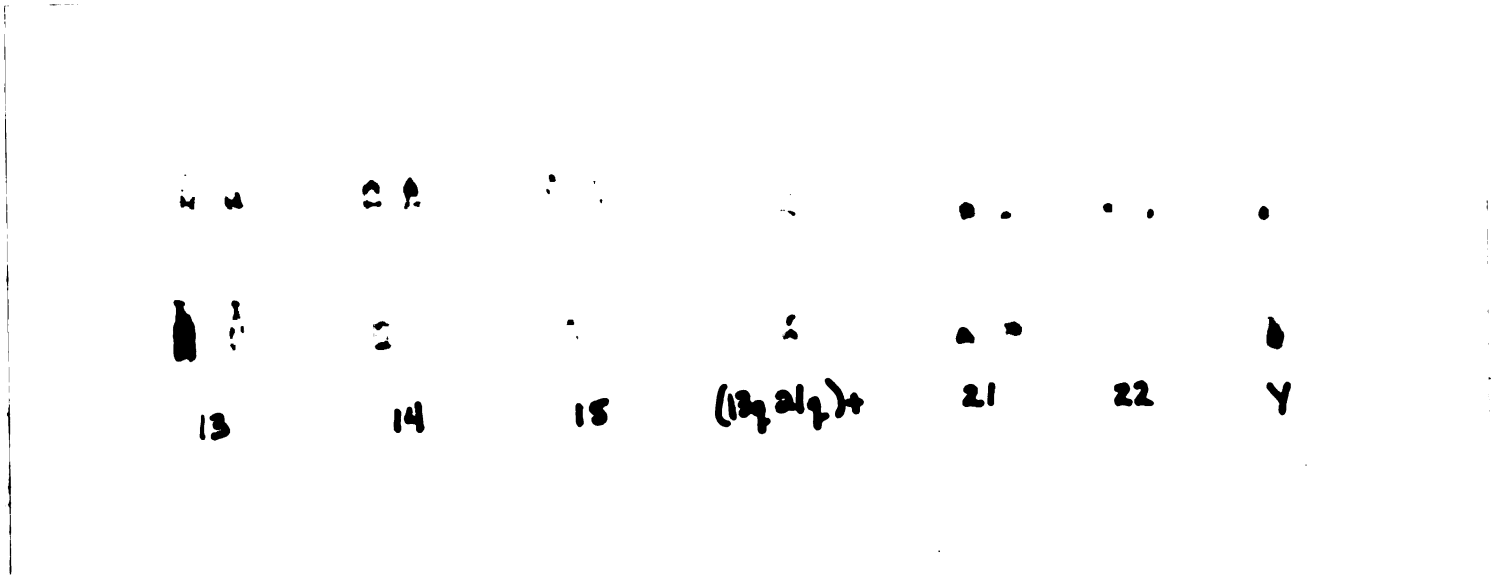

Figure 14b. Case no. 13, partial karyotype.

included small stature, possible microcephaly, seizures and severe mental retardation.

Routine chromosome studies revealed a 47 count with an extra acrocentric chromosome smaller than a D group, but larger than a G group. His father and younger brother were karyotypically normal. His mother and younger sister, M. H. and A. H. respectively, carried balanced translocations. They each had 5 normal D group chromosomes, 3 normal G group chromosomes, one deleted D and the acrocentric intermediate in size between a D and G. Both M. H. and A. H. are asymptomatic with a cytogenetic diagnosis of 46,XX,Dq-,G-,t(DqGq)+. After determining the rearrangement in his mother and sister, P. H. was thought to be 47,XY,t(DqGq)+.

Giemsa banding on A. H. and M. H. revealed one normal no. 13, one normal no. 21 and one deleted distal portion of the long arm of a no. 13. The break in the no. 13 appears to have taken place between the two distal dark bands on the long arm. The acrocentric intermediate between a D and G was shown to be a translocated chromosome displaying almost all or perhaps the entire no. 21 in the centromeric position with the distal band of the long arm of a no. 13 attached to the no. 21,46,XX,13q-,21-,t(13q21q)+. Thus, the translocated chromosome appears darkly banded at the centromere followed by a clear unbanded region and a heavy distal band.

The banding patterns on P. H. revealed 46 normal chromosomes plus the acrocentric which was shown to be the

same translocated chromosome present in his mother and sister, 47,XY,t(13q21q)+. Thus, he is partially trisomic for the distal portion of the long arm of no. 13 and partially or completely trisomic for a no. 21.

Case #14: J. K. born 8-?-69 (See Figures 15a and 15b)

This child, the youngest of 3 pregnancies (the second ending in a full term stillborn), displayed a saddle nose, syndactly of the fourth and fifth fingers of the right hand, low set ears, high arched palate and gross motor and mental retardation.

Routine chromosome analysis showed a complex de novo chromosomal rearrangement involving chromosomes of the A1, B and C groups. It was postulated that a double translocation had taken place with the short arm fragment of the C going to either the no. 1 or the B and the long arm fragment going to the opposite chromosome, 46,XY,t(1q+,Bp+,Cp-q-).

Giemsa banding studies confirmed a double translocation involving chromosomes 1, 5 and 8. It appeared from the position of the bands that there were two breaks in chromosome no. 8, involving approximately half of each arm, the long arm fragment translocating to the proximal arm of no. 5 and the short arm fragment to the distal arm of no. 1, 46,XY,t(1q+,5p+,8p-q-). Giemsa karyotypes on both parents were normal.

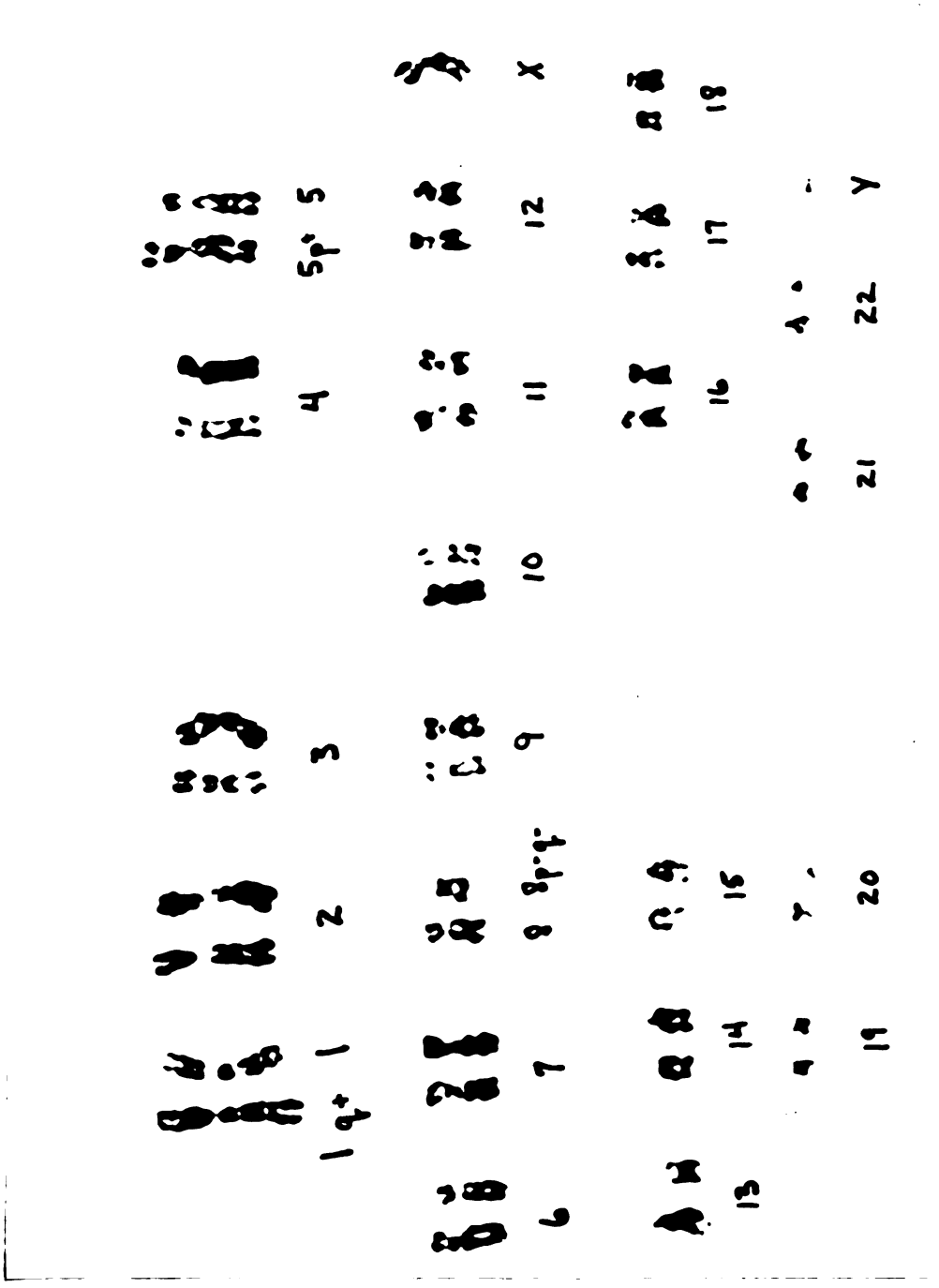

Figure 15a. Case no. 14, 46,XY,t(8p-q-,1q+,5p+), complete karyotype.

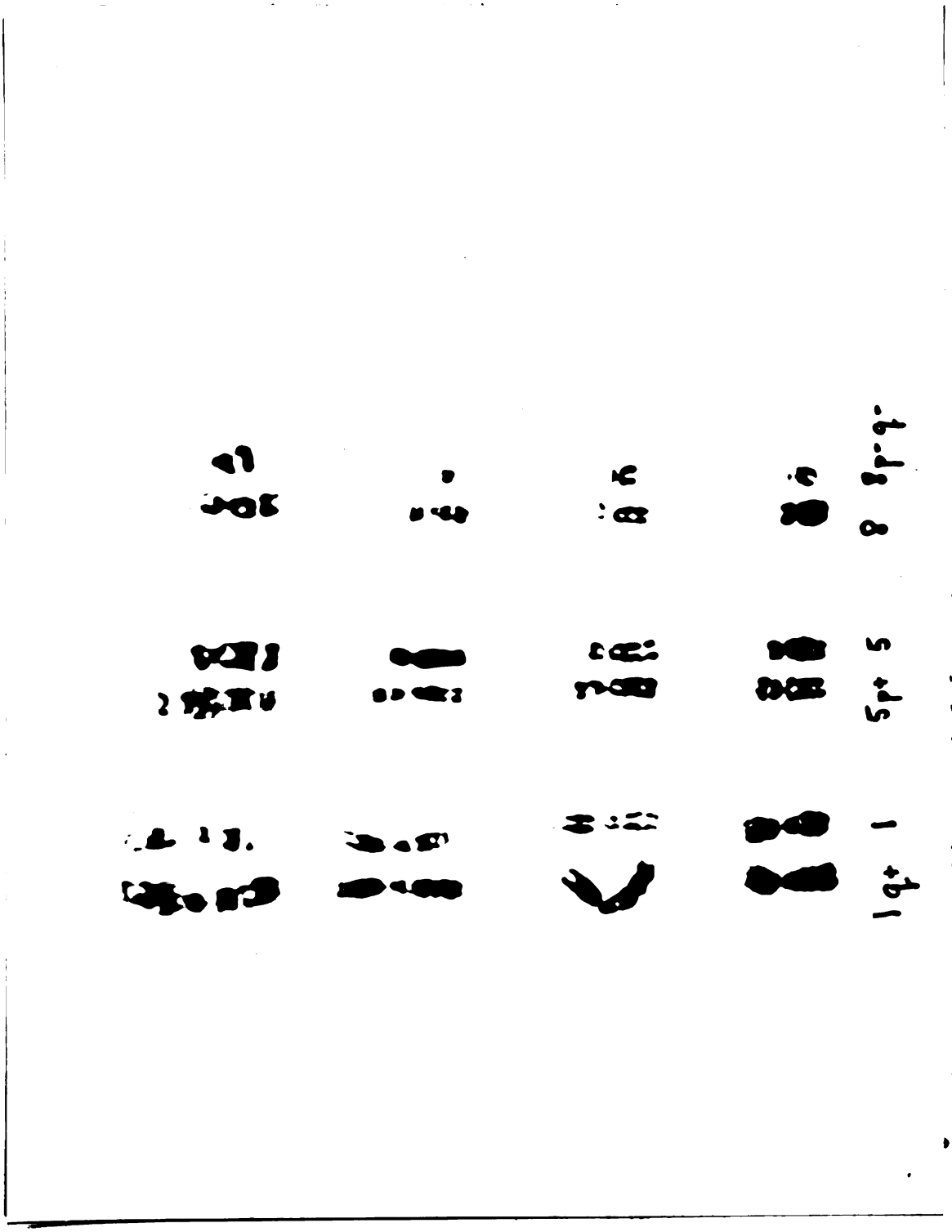

Figure 15b. Case no. 14, partial karyotype.

DISCUSSION

Classical karyotype analysis can often identify gross chromosomal rearrangements, but in most instances fails to accurately identify the individual chromosomes involved. Autoradiography with tritiated thymidine has extended identification to groups B, D and E, but it requires time and is often inconsistent. Furthermore, it does not permit classification of C, F and G group chromosomes, except for the late replicating X.

The relatively new techniques of Giemsa banding and fluorescence have made it possible to visually distinguish all of the chromosomes in man. The Giemsa method, however, is more advantageous than fluorescence since it involves no special microscopic equipment and results in permanent preparations. The Giemsa also produces clearer bands making it easier to identify possible break points in the chromosomes involving translocations or deletions.

Whereas the fluorescence bands are consistent and well agreed upon, the individual Giemsa methods vary in both the clarity and number of bands. Crossen (1972) feels this is probably a result of the different procedures used. Figure 16 displays the banding patterns reported in four denaturation-renaturation techniques (A-D), the Seabright trypsin

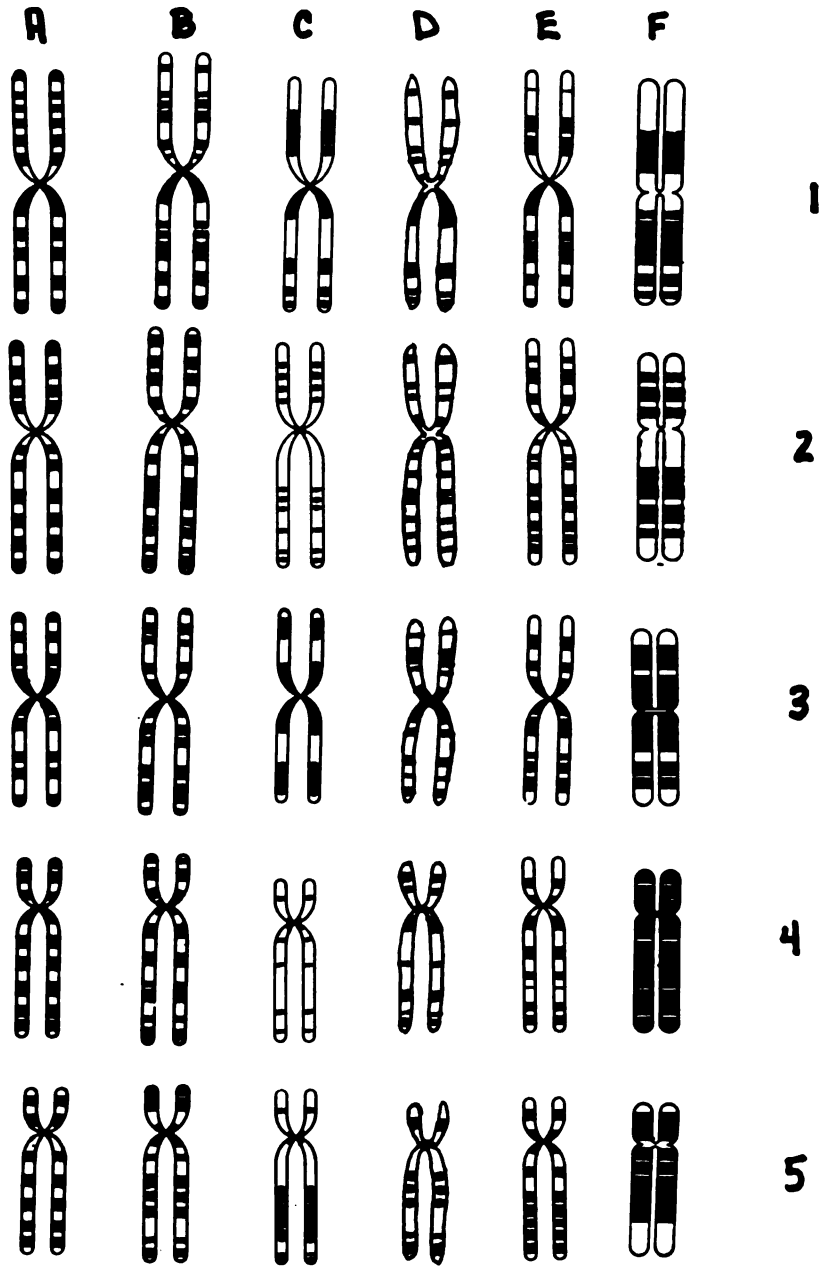

Figure 16. Comparison of banding patterns by 6 different techniques.

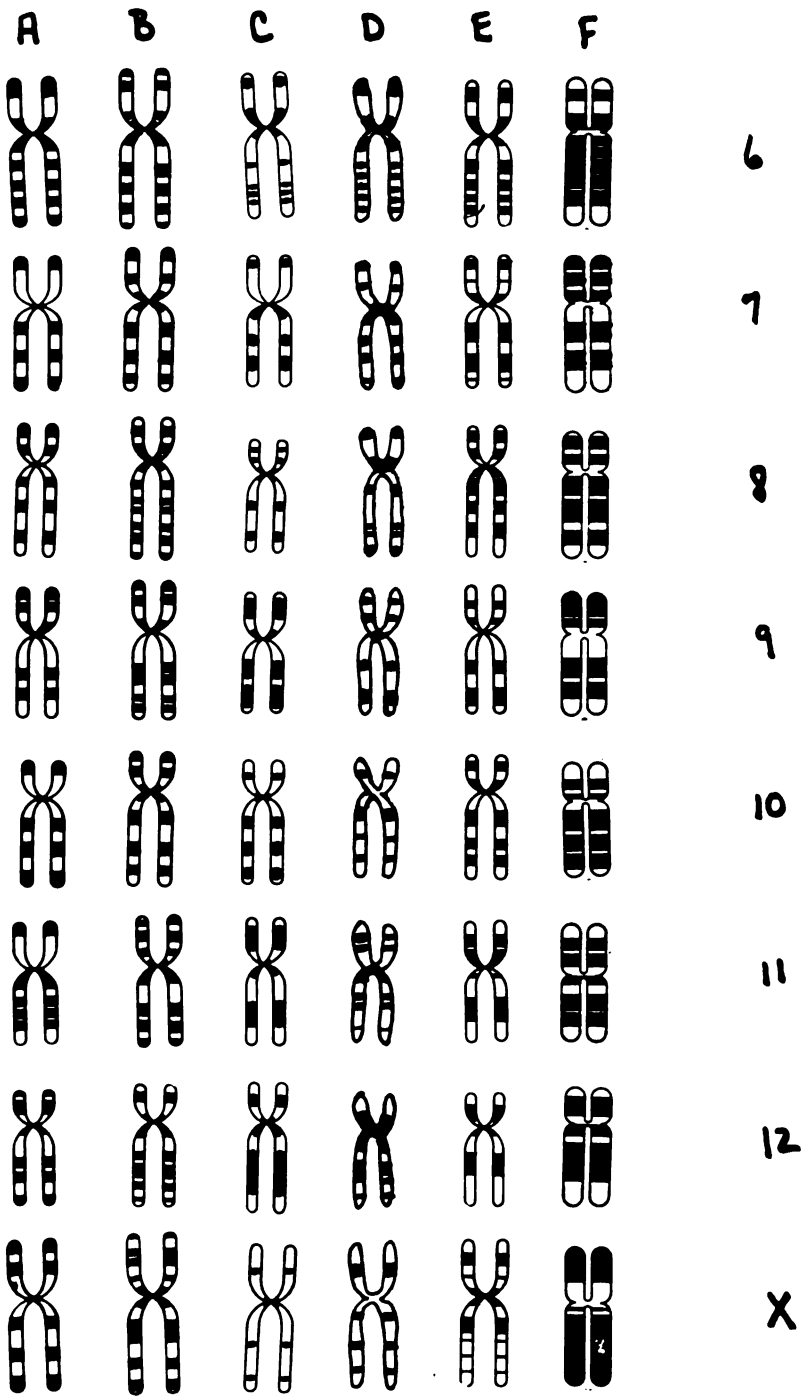

Figure 16. Continued.

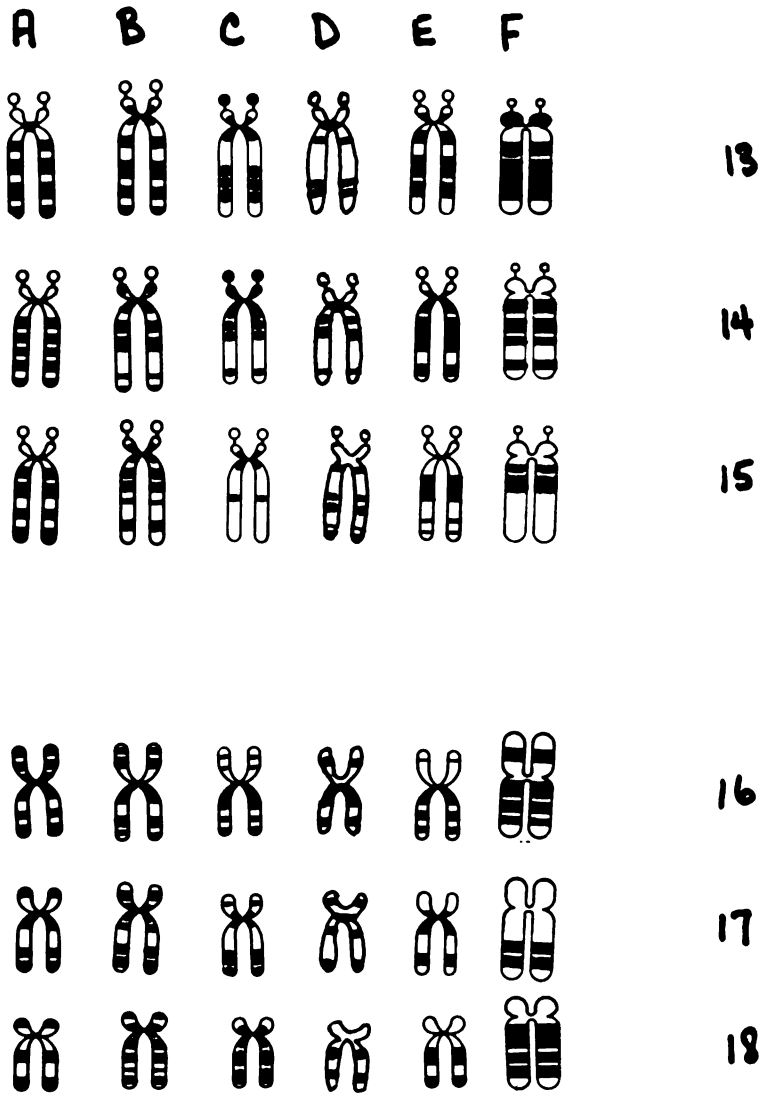

Figure 16. Continued.

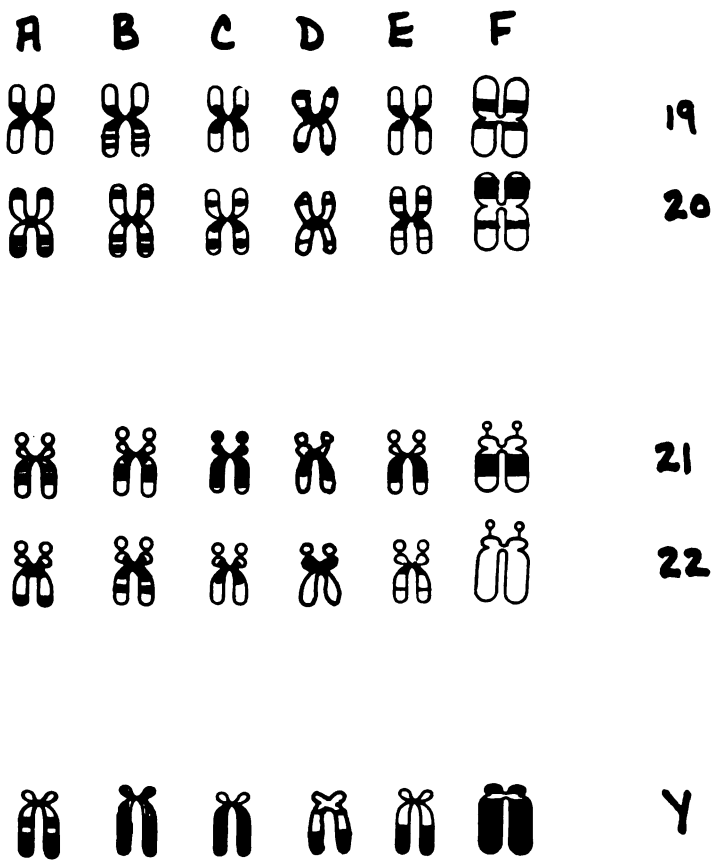

Figure 16. Continued.

procedure (E), and the fluorescence procedure (F), [A. Crossen (1972); B. Schnedl (1971b); C. Drets and Shaw (1971); D. Sumner et al. (1971); Seabright (1972); F. Lin et al. (1971)]. In the larger chromosomes, especially nos. 1,2,4, and 5, the most noticeable differences exist. Nos. 16,17,18, 19,20,21 and the Y are the most consistent in all of the procedures.

In the incubation techniques (A through D), the denaturation pretreatment is an important factor with NaOH being necessary for the production of clear bands (Crossen, 1972). Also the length of time and the range in temperature in the renaturation buffer might account for variations. It appears that a longer incubation time results in fewer bands since Drets and Shaw (1971), who utilize a 72 hour incubation period, report fewer bands than other authors. The common choice of temperature for reassociation is 60°C, but higher temperatures have been reported (Dutrillaux and Lejeune, 1971). Perhaps this may account for the reverse banding patterns obtained by these authors.

The trypsin bands of Seabright (E) agree most favorably with the quinicrine bands (F). In the 10 controls studied, there were no outstanding variations in banding patterns, except for the X chromosome. Staining intensity, however, did vary. A larger sample of normals may have revealed polymorphisms, which could lead in time to complete mapping of human chromosomes. Pearson (1970) has reported two fluorescent

types of chromosome 3 in the general population and found that while three-quarters of chromosome 3 have one pattern of fluorescence, one-quarter has a different pattern. Enlarged satellites, asymetry of chromosome no. 1 and differences in the length of the Y chromosome have been observed in routine karyotype procedures and are usually inherited (Cooper and Hernits, 1963; Cooper and Hirschhorn, 1964; Makino et al, 1963). Craig-Holmes and Shaw in 1971, described seven heterochromatin variants among four normal individuals, utilizing centromeric banding techniques. These findings led them to conclude that these variants occur with a much higher frequency in the population than would be expected. Crossen (1972), found a man with atypical banding of the Y chromosome via his denaturation-renaturation procedure.

It was also noted in the controls of this study, that the size of the chromosomes, particularly in the C6-12 group, displayed some variations. The most striking example was that no. 11 and no. 12 were quite frequently larger than no. 8 and no. 9. This finding has not been reported elsewhere, but is suggested by the data of Drets and Shaw (1971). They originally classified the C group chromosomes by size and mislabeled no. 12 and no. 11 as no. 8 and no. 9 respectively. They later changed their findings in order for their chromosome banding patterns to conform with those of fluorescence.

Another obvious size variation occurs in the G group. The Y is easily distinguished not only by its intense distal band, but also by its morphological appearance. No. 21, however, is consistently smaller than no. 22. This discovery led at least one author (Ridler, 1971), to recommend that Down's syndrome be known as trisomy 22, since the cases of Down's syndrome show trisomy for the smaller class of G-group chromosomes.

Size variations were also apparent in homologous pairs. This type of information would provide a useful link in determining which heritable characteristics of the chromosomes came from which parent.

The optimal resolution of bands was obtained from elongated chromosomes of early metaphase. In contracted chromosomes the finer bands tend to merge together, so that only the main bands are prominent. The main bands in no. 1, for example, are in the medial portion of the short arm, just beneath the centromere, and in the medial portion of the long arm. The main bands of the trypsin procedure (Figure 16E) agree with the main bands of the ASG technique (D) of Sumner et al. (1971).

The centromeric regions of nos. 3,6,11,12,16,19 and 20 are consistently deeply stained. In a normal female with two X chromosomes, both of the X's band in the same manner and it is not possible to distinguish the late replicating X

chromosome. If the bands truly do represent heterochromatic regions, one would expect the late labeling X to be intensely stained. This has not been found.

Case 1

In each of the 14 patients analyzed, fluorescent banding results agreed with the Giemsa.[1] There appear to be no consistent physical findings associated with the chromosomal abnormality of triple X females. There does seem to be an increased frequency of mental retardation, which is also displayed in case #1 (Kohn et al., 1968). Two of the 3 X chromosomes in K. H. appear in both size and position of the bands, identical. If her parents were to be karyotyped using the Giemsa banding procedure, it should be possible to determine in which parent the non-disjunction occurred. It would have had to take place in meiosis II in either parent since two of the X's are identical. As stated previously, the only polymorphic banding patterns found were in the X chromosomes. If the non-disjunctional error had occurred in the first meiotic division of her mother, one would have expected 3 different X chromosomes.

Some XXX females are fertile and the lack of XXX and XXY children in their progeny indicates preferential

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[1]Fluorescent banding patterns used in the following comparisons were performed by S. Cullen (1972).

segregation with two X chromosomes going to the polar body and the balanced oöcyte developing into the ovum (Miller, 1964).

Cases 2 and 3

In the cases with sex chromosomal aberrations, especially where the Y chromosome was involved (cases 2 and 3), the fluorescence proved to be the more reliable procedure, since the Y fluoresces brilliantly and can be easily detected. Case 3 was especially interesting since he seemed to fit more into the category of XYY, both physically and socially, than into the classification of Klinefelter's syndrome.

Case 4

An isochromosome involves a single transverse replication of the centromere. As a result the long arms of sister chromatids become fused as well as the short arms. Since the short arm appears to contain the genetic material necessary for sexual development, all patients with an isochromosome for the long arm of the X chromosome would be expected to fulfill the diagnosis of Turner's syndrome (Bartalos and Baramki, 1967). Aside from extreme short stature which may be due to her severe scoliosis, case #4 only displays a wide carrying angle which is consistent with Turner's syndrome.

As would be expected, both arms of the isochromosome were identically banded, with 3 bands in each arm and an unbanded centromere. It was noticed that when photographic spreads containing less than 46 chromosomes were analyzed, the isochromosome X was consistently absent. This same finding was also evident in karyotypes made from routine staining methods. This might be due to abnormal segregation of the iso-X during mitosis. Thus, K. P. is very likely to be a mosaic, 45XO/46X iso $\bar{x}$.

Case 5

While the autosomal deletions studied could have been detected through autoradiography, the Giemsa and fluorescent procedures were easier and more consistent. Routine karyotype analysis would not have been able to distinguish which of the two B group chromosomes was deleted in case #5, since there is also a syndrome which occurs with the deletion of a no. 4, Wolf's syndrome, having similar physical findings without the cat cry. Giemsa staining, however, easily identified the deleted chromosome as a no. 5 by its characteristic broad band in the medial portion of the long arm.

As other syndromes due to chromosomal anomalies, Cri-du-Chat shows variations that could be explained by differing losses of genetic material from the short arm of no. 5. The leading signs in addition to the characteristic cry are microcephaly and mental retardation. The patient in case #5

appears to be missing almost the entire short arm, approximately 60-75% lost. This may account for his more severe clinical picture including micrognathia, epicanthal folds and bilateral simian crease.

Case 6

Loss of the short arm of chromosome no. 18 was the first autosomal deletion observed in man. Because of the small number of patients with this type of deletion, there are no sufficient clues for establishing a clinical diagnosis. A number of malformations appear to be common to several patients including strabismus, ptosis, epicanthus, hypertelorism, low set ears, flat nose, tooth decay, micrognathia, web neck, short fingers and a high ridge dermatoglyphic count (Warkany, 1971).

Case #6 has no unusual physical findings except strabismus and tooth decay. Banding specifically identified the E group chromosome in question as a no. 18, missing the entire short arm with two prominent bands in the long arm.

Since this initial study, another female with mild retardation, conductive deafness and a short neck due to unspecified spinal cord abnormalities, has been found to display an E deletion which is believed to be a no. 18. Warkany (1971), has shown that there are more reported cases of affected females than males with a short arm deletion of an E 18. The only consistent finding present in both of the

females detected at the Lapeer State Home and Training School is a low I.Q. Dermatoglyphics have not been done and it would be interesting to see if there really is an elevated ridge count as reported in the literature.

Cases 7-9

In the chromosomal anomalies occurring in members of the G group, Giemsa banding and fluorescence are the only techniques which can individually distinguish the chromosomes involved. For this type of study, they become exceedingly important.

In each of the three cases analyzed, involving G/G translocations, Giemsa banding identified the error as a 21/21 fusion. The translocated chromosome resembled by size and centromere position, a member of the F group. By banding patterns, however, it was obviously two no. 21's, since it appeared entirely stained.

Case #7 was the product of two karyotypically normal individuals and must have arisen de novo during embryogenesis. Cases 8 and 9, however, had a father who was a translocation carrier. The reproductive history led to the assumption that he was a 21/21 carrier, since there were no normal children in the progeny. Theoretically, it would be possible to distinguish which type of translocation he carried, by a testicular biopsy. If during meiosis at diakinesis, there were 22 bivalents (including the X and Y) and one

univalent, it would be assumed that the father was a 21/21 translocation carrier. If there were 21 bivalents (including the X and Y) and one trivalent, it would be assumed that he was a 21/22 translocation carrier. Prior to the Giemsa banding technique, this was the only method available to accurately state the risk values for a carrier father. If, however, one of the parents was found to carry the translocation and if they had normal offspring, or if one of the children was a balanced G/G translocation, the probable diagnosis would be a 21/22 translocation. These have a fairly low risk, perhaps less than 10%, of recurrence (Bartalos and Baramki, 1967).

Case 10

Hecht et al. (1968) studied by autoradiography 20 individuals with Down's syndrome due to 13-15/21 centric-fusion translocations. They found no cases where chromosome 13 was involved, 18 cases involving chromosome 14 and 2 cases involving chromosome 15. The differences observed from those expected were highly significant indicating that the 13-15/21 translocation is nonrandom. They felt that this nonrandomness might reflect different tendencies for the broken D group chromosomes to fuse with no. 21, perhaps because of spatial relationships within the nucleus. They also suggested that the nonrandomness might reflect differences in

the frequencies with which chromosomes 13, 14, and 15 break near the centromere, possibly due to differences in molecular organization.

In 1970, Capoa and Rocchi demonstrated autoradiographically the first 13/21 translocation over a published total of 40 cases. This type is of very low frequency.

Hecht and Kimberling (1971) extended the autoradiographic studies of the D group chromosomes involved in t(DqGq), listing 64 cases involving chromosome 14, 9 involving chromosome 15 and only 2 involving chromosome 13. These cases included all of those reported in the literature up to that time. They favored the concept that perhaps all Robertsonian rearrangements form from an orderly, nonrandom process such as meiotic pairing and exchange, rather than from random breakage and fusion.

The D group chromosome involved in case #10 was shown from its band near the centromere and a band near the distal end of the long arm to be a no. 14. This is in agreement with the literature since most of the D/G translocations reported have been of the 14/21 type. The banding procedures are extremely advantageous in detecting these types of translocations, since the D group chromosomes are easily distinguished allowing for more exact results. Quite often, autoradiography shows discordant labeling patterns (Hecht et al., 1968).

Case 11-13

The more complex translocations studied revealed some interesting results. This was where the Giemsa technique was especially useful since the fluorescence could not adequately identify the break points within the chromosomes. The bands themselves, were also superior to the Q bands.

Case #13 is interesting in that the trisomy D clinical features seem to mask the trisomy 21 traits. It has been previously reported that the major trisomy D congenital malformations including ocular defects, cleft palate and polydactly, are carried on the distal portion of the long arm (Gerald and Bloom, 1968). These findings are reinforced with the present case, since P. H. is trisomic for the distal portion of the long arm of chromosome 13. Other signs often seen in D trisomy are elevated fetal hemoglobin levels and increased neutrophil projection counts. Gerald and Bloom have suggested that these traits are located near the centromere regions of chromosome 13. P. H., therefore, would not be expected to exhibit these findings. Unfortunately, these tests have not been conducted.

Theoretically, both M. H. and A. H. (cases 11 and 12), who are balanced translocation carriers, have the possibility of producing two other types of abnormal offspring, which could happen 50% of the time (See Figure 17). These would include an individual trisomic for the distal portion of the long arm of no. 13, who would be expected to have the

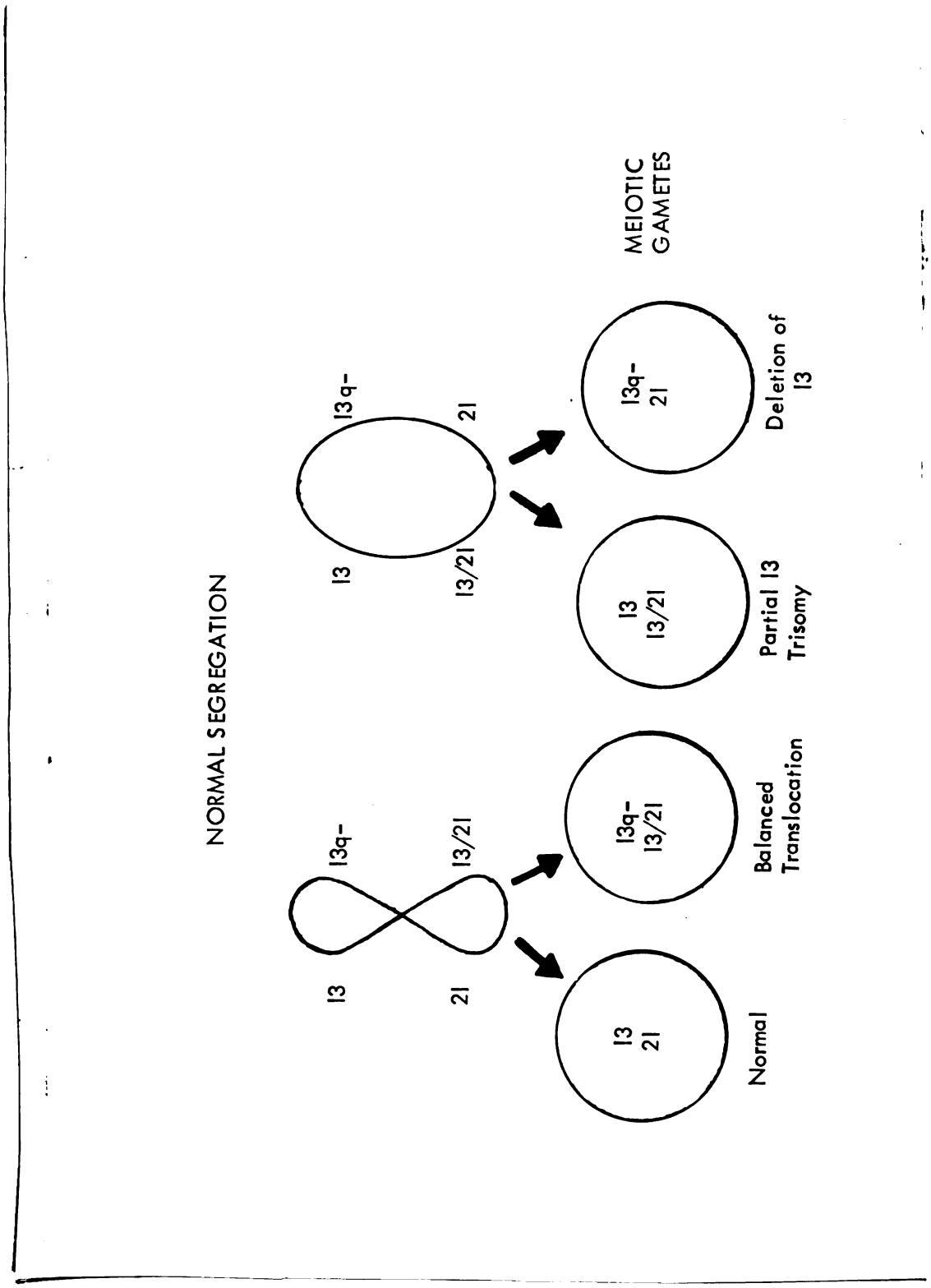

Figure 17. Normal segregation in cases no. 11 and 12.

phenotype of a D trisomy, and an individual with a deletion of the distal portion of the long arm of no. 13, which would probably be lethal. If nondisjunctional errors such as P. H. are also included, the probability of having an abnormal child becomes greater than 50%. Dekaban et al. (1963), noted an extremely high frequency of familial chromosome abnormalities in an unselected group of individuals with Down's syndrome. These findings led them to suggest that "minor" abnormalities may increase the frequency of nondisjunction of other chromosomes during meiosis. Thus, it is possible that the structural aberration displayed by A. H. and M. H. may have an effect on causing abnormal segregation more frequently than would be expected.

Since it is known that P. H. has inherited the translocated chromosome from his mother, a more exact cytogenetic diagnosis in his case would be 47,XY,t(13q21q)+ mat.

Case 14

Even though the patient in case #14 exhibited severe mental and motor retardation, there was no evidence that the translocations were unbalanced. The possibilities of minor deletions, however, cannot be excluded. Francke (1972), also noted apparently balanced chromosomes states in patients with abnormal phenotypes. She hypothesized that the position effect, a well established phenomenon in Drosophila, might account for the abnormalities in patients with a full

genetic complement. If this is true, it becomes apparent that the full potential of a gene is dependent on its neighboring genes.

The findings also suggest, contrary to accepted dogma, that reciprocal translocations have not occurred. There has been no apparent loss of chromosomal material on the ends of chromosomes no. 1 and no. 5. These observations agree with those of Francke (1972), and would lend further support to her suggestion that the unbroken chromosome tips may normally be "sticky." It is impossible to make conclusions based on only one case and more Giemsa studies will have to be conducted.

Figure 18 shows the approximate points where the two breaks on chromosome no. 8 have occurred and the method in which these segments have translocated to nos. 1 and 5. The double translocation in this patient must have arisen de novo during embryogenesis since both of his parents were shown to be normal by the banding techniques.

There are two other cases of double translocations reported in the literature (Nuzzo et al., 1968; Fredga and Hall, 1970). In each instance, the chromosomes involved and the physical stigmata were different from the present case. One of these (Nuzzo et al., 1968) also reported a de novo chromosomal rearrangement where both of the parents were karyotypically normal. The other (Fredga and Hall, 1970), showed the mother of the affected individual to carry a balanced chromosome translocation.

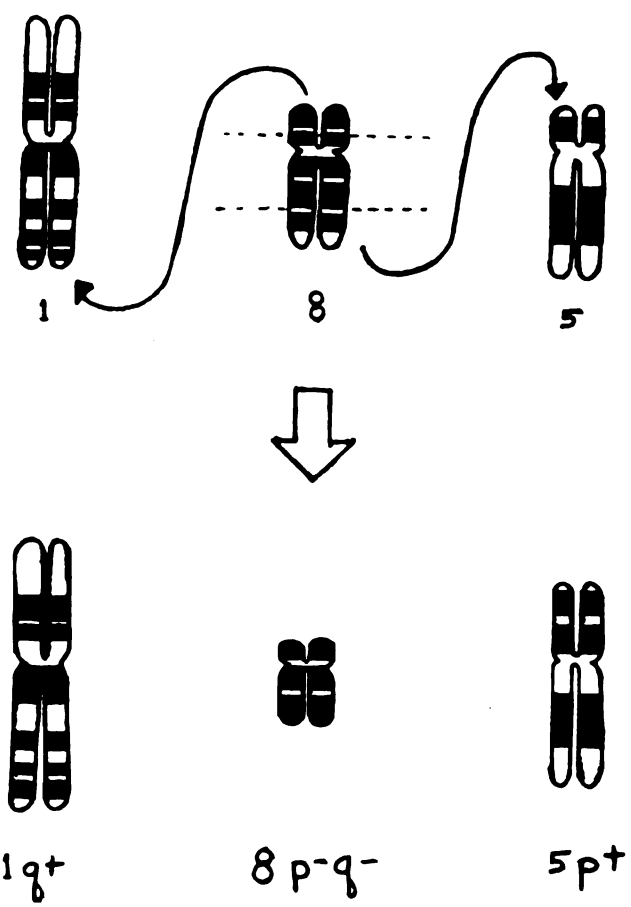

Figure 18. Idiogram of translocated chromosomes of case no. 14.

Problems Encountered in the Trypsin-Giemsa Method

The trypsin-Giemsa method did present some disadvantages. Its major fault was in the trypsinization which ranged from 15 seconds to one minute, depending upon the individual. The technique required experimentation and often 3 or 4 slides were needed in order to determine the optimum time for enzyme digestion. If the slides were exposed to the trypsin for an extended period, the chromosomes were destroyed. If they were not immersed sufficiently, the chromosomes were not banded. Refinement of technique, however, should overcome these initial inconsistencies.

CONCLUDING REMARKS

Of the variety of G band techniques available, the procedure of Seabright which uses the proteolytic enzyme trypsin, appears to be the fastest and most consistent. It is now possible to identify all of the homologous chromosome pairs in man.

The technique, being a recent addition to human cytogenetics, is far from being perfected. While the bands do occur and do allow identification of translocations and deletions, they are not always as clear and precise as one would like. They are, however, more distinct than the bands produced by fluorescent dyes and allow for a more probable identification of the break points within the chromosome.

It will be interesting to see in the coming years, whether the technique will be transcended to such an extent that it will be possible to recognize fine structural rearrangements which might otherwise go unnoticed by the classical method of chromosome identification. Such a procedure would be of incalculable aid to the cytogeneticist and genetics counselor.

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APPENDIX

APPENDIX

To make 10xGKN working stock solution

80 gm. NaCl

4 gm. KCl

10 gm. glucose

1000 ml. distilled H_2O

Dissolve and add 3-4 ml. chloroform.

Refrigerate.

To make 0.25% trypsin - GKN

.25 gm. powdered trypsin

10 ml. 10xGKN

90 ml. distilled H_2O

Dissolve and freeze into 10 ml. alliquots since the trypsin loses its activity if left at room temperature for any extended period of time.

The trypsin is most active in a temperature range of 24°-37°C. Thus, it should be thawed thoroughly to room temperature before use.

It was found that fresh trypsin solution was not as reactive as that which was first frozen and thawed before use.

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