

CHAPTER 11

Chromosome Analysis

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The early history of the development of cytogenetics is described well in the book by T. C. Hsu (1), beginning with the first drawings of human chromosomes published in 1879 by Arnold and the many attempts made to count the number of chromosomes present in any cell (attempts by

Hansemann in 1891, DeWiniwarter in 1912, Painter in the 1920s, and Koller in 1937). The published pictures from those times show tangled mats of chromosomes, and it is quite clear why there was so much difficulty in deciding on the correct number. With improved techniques, in 1956 Tijo and Levan determined 46 to be the correct number for normal human cells. Shortly thereafter, the discovery of different chromosome constitutions in persons with recognized syndromes (trisomy G in Down syndrome described by Lejeune in 1959 and trisomy D in Patau's syndrome in 1960) led to the understanding that many abnormal human

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conditions might be caused by abnormal chromosome constitution; this started the search for clinical applications of the new knowledge. The wide scope of those clinical applications can be appreciated by reviewing the areas in which cytogenetic analysis is currently very useful. These can be divided into two major groups, the first applying to constitutional (or congenital) conditions and the second to acquired conditions.

The clinical investigations of constitutional chromosome status include prenatal screening for chromosome disorders, differential diagnosis of congenital anomalies, antenatal sex determination, investigation of primary amenorrhea, investigation of male or female infertility, habitual abortion studies, genetic counseling of parents of congenitally abnormal infants, and investigation of mental retardation owing to fragile sites. Studies of acquired chromosome disorders include differential diagnosis of malignancies, identification of malignant and benign tumors, cancer staging (and diagnosis of remission or relapse conditions), bone marrow transplantation (comparison of donor and host cell chromosomes), and studies of mutagens and carcinogens and their effects on cells.

For all of these different applications, the basic approach is the same. The cells are arrested in metaphase or prometaphase, treated to obtain readable metaphase spreads, and then counted, karyotyped, and analyzed. The number of cells to be examined is determined by the nature of the tissue and the study being undertaken, following the standards and guidelines published by the American College of Medical Genetics (ACMG) (2).

This chapter deals with the ultimate goal of cytogenetics: chromosomal analysis and the identification of the various types of abnormalities that may be encountered during this process. This is documented through the construction and interpretation of the pictorial karyotype, an orderly arrangement of chromosomes from a single cell examined in the metaphase or prometaphase state.

The first section covers the basic terminology for chromosomes and introduces the counting and karyotyping procedures. The conventions for grouping the chromosomes by size are discussed as well as the differences between the effects achieved by homogeneous stain and by the various banding techniques. There is a brief discussion of the methods used to assess the fine detail observable in the banded chromosome preparation.

The second section discusses the components of a complete study. These are specified by the ACMG and the College of American Pathologists (CAP), which regulates the laboratory with yearly inspections. Technologists are very much aware of the possibility of mosaicism and of the need to prevent errors in analysis or reporting of cases. There is also great emphasis given to quality and maintenance of records. The work that is produced must be stored so that it is available at a future date.

The third section discusses the various types of aneuploidy that have been reported for the human karyotype

and gives a brief description of the clinical significance of these aberrant karyotypes. The section continues with a discussion of the various types of rearrangements that are the result of chromosome breakage and introduces the terminology related to breakage and rearrangement. After describing the various banding patterns of the individual chromosomes, the chapter offers brief descriptions of some of the major abnormalities related to gain or loss of parts of each chromosome.

This chapter also gives a brief overview of the system of nomenclature that has been developed to describe the chromosome sections so that verbal descriptions of variations and rearrangements can be used. This permits international understanding of these variations.

Much of the information used in compiling this chapter can be found in several general textbooks (3–10); choose the most recent editions for further reading. There are also books giving details of procedures (11–13), and catalogs of genetic conditions (14), as well as papers from the literature illustrating the special points made in the chapter. New material revealing further insights is constantly appearing. The field of genetics is changing quickly and results of research are always being published. Regular reading of current journals is strongly recommended for anyone who hopes to keep up to date in this field.

CHROMOSOMES AND THE METAPHASE SPREAD

In order to analyze chromosomes it is necessary to know how chromosomes look, to be able to identify them, and to recognize abnormalities. To obtain a knowledge of chromosome shape and size we begin by looking at a photograph of a metaphase spread taken from a preparation stained directly with Giemsa stain, without the use of any banding techniques (Fig. 1). Most laboratories have abandoned nonbanded (also called solid, conventional, routine, or homogeneous) staining and work only with banded preparations because banding permits positive identification of individual chromosomes and elucidation of band locations of breakages and rearrangements. However, it can be helpful to look at nonbanded metaphases (a few of these can usually be found on any trypsin-banded slide). Nonbanded spreads are easier to count and can be analyzed for the presence of correct groups of chromosomes, even though individual chromosomes cannot be identified. Chromosome morphology is more easily seen on nonbanded preparations, particularly the terminal ends of chromosomes, which tend to become unclear with trypsin banding. Many numeric and morphologic abnormalities can be observed in nonbanded preparations when they may not be detectable by G or even Q banding, including multiple satellites, bisatellited markers, fragile sites, other breaks and gaps, and double minutes.



FIG. 1. A: Routine metaphase spread, homogeneous stain. B: Karyotype of metaphase spread.

The metaphase to be analyzed should have chromosomes that are well separated from each other but that are clearly from the same cell. It is best if there are no overlaps; if overlaps are present, it must be possible to distinguish each chromosome. The nonbanded chromosomes should be evenly stained and quite dark, and the morphologic features such as arm length and separation should be clear.

Terminology

In any new science, special terms have to be developed in order to facilitate discussion (14). Some of these terms are discussed here, and they are illustrated in Fig. 2. The metaphase spread is the collection of chromosomes from one cell visible in the condensed form that is assumed during cell division. Harvest and slide making techniques have been developed to enhance the number of cells in metaphase so that sufficient spreads are available for examination. Studying the spread at high magnification shows that chromosomes come in different shapes and sizes, from quite large to very small.

At metaphase, each chromosome is composed of two chromatids joined together at the primary constriction, which is usually called the centromere. The sections of the chromosome on either side of the centromere are called arms. In any metaphase spread of human chromosomes, some chromosomes have their centromere in the middle, some have it at one end, and some have it some-

where between these two points. The terminology of chromosomes reflects these differences. Chromosomes are described as metacentric (centromere in the middle), submetacentric (centromere a little away from the middle so that one arm is definitely longer than the other), acrocentric (centromere close to one end), or telocentric (centromere right at the end). There are no truly telocentric chromosomes in the normal human karyotype. In order to discuss sections of chromosomes easily, short arms are named p arms (for *petit*), and long arms are called q arms, in the shorthand used for describing karyotypes.

Every metaphase chromosome must have a centromere to attach to the spindle at cell division. Some chromosomes also have a secondary constriction, which is a thin, thread-like portion that does not stain very dark with Giemsa stain. Secondary constrictions frequently appear on the short arms of acrocentric chromosomes, connecting the satellites (very small pieces of DNA) to the centromere. The secondary constrictions on the acrocentric D and G chromosomes have been shown to be the site for synthesis of ribosomal material in the interphase nucleus and are termed the nucleolus organizer regions (NORs). Secondary constrictions are sometimes seen on other chromosomes, such as chromosome 9, just below the centromere. On photographs, secondary constrictions may be confused with gaps because they are so thin and pale; they can be seen through the microscope, however, and may thus be differentiated from true gaps.

Counting the Metaphase Spread

It is necessary to determine the number of chromosomes in any metaphase spread by counting. A technologist counting directly through the microscope commonly uses a mechanical counter to assist in keeping track. Counting the chromosomes is a relatively simple task, and most technologists develop a habitual method of counting, such as always starting in the same quadrant, counting by twos, or mentally splitting the whole collection into several smaller groups. Counting from a photographic print on which one can make marks is a good way to ensure that each chromosome is counted. In the beginning it may be necessary to count each spread more than once to be sure of accuracy.

Several cells must be counted to determine the modal number, or the most common (predominant, or characteristic) total of chromosomes in each metaphase of a particular sample. Individual cells may vary from the modal number because of random loss or gain of chromosomes in slide making. The normal modal number for a human is 46, which is termed the diploid complement and is formed by the joining of two haploid cells (gametes) at fertilization. This number can vary through addition or subtraction of chromosomes in gametogenesis, fertilization, or embryogenesis, which affects the whole constitu-

There are three basic shapes of human metaphase chromosomes depending on the location of the centromere

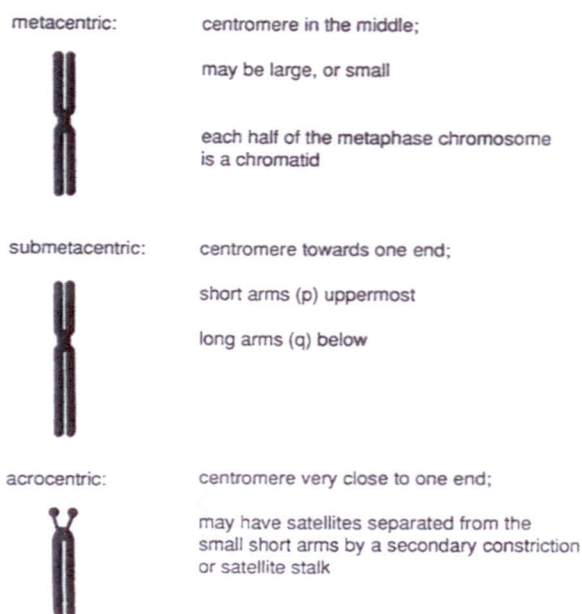


FIG. 2. Chromosome morphology and terminology.

tion of a person. The number can also be altered within some particular cells of the body, leading to mosaic cell lines in the newly formed individual; some types of cancer also have altered modal numbers as one of their characteristics. Variations in constitutional modal number from 45 to 49 have been reported. Cells that have other than the normal diploid complement are termed aneuploid. If fewer than 45 chromosomes are present in the metaphase, it can be assumed that some have become lost in the processing and that the metaphase is unsuitable for analysis, unless there is reason to suspect the presence of cancer cells.

Sometimes cells are aneuploid by complete haploid sets of chromosomes; they are then called polyploid. If the total count is 69 the cell is triploid; triploidy occurs rarely as an abnormality and is rapidly lethal. A more frequent occurrence in tissue culture samples is the presence of tetraploid cells (cells with 92 chromosomes). Mosaicism with diploid and tetraploid cell lines is possible *in vivo*, but is extremely rare, and the tetraploid cells found *in vitro* are generally artifacts of the tissue culture process. Tetraploidy is not unusual in normal bone marrow and is probably due to dividing megakaryoblasts or megakaryocytes. Tetraploid cells are seen occasionally in blood culture analysis and may be indicative of an aberrant cell line in the patient, particularly with cancer patients. Some solid tumors have a tendency to develop tetraploid clones in culture.

Making the Karyotype

The word karyotype originally meant just the arranged picture of the chromosomes of a single cell, but with time the meaning has expanded to also mean the written description of the picture because the two are both descriptions of the same thing. Here we will discuss the principles used in the making of the pictorial karyotype, and in the nomenclature section we will continue with the procedure for writing descriptive karyotypes. The pictorial karyotype is an orderly arrangement of the chromosomes according to international conventions (14–17). The chromosomes may be cut from a photographic print and arranged on a preprinted form using various types of glue or tape to affix the chromosomes permanently to the sheet. Unless a karyotype is needed for publication, the simplest way to attach the photos is to cover the chromosomes with nonyellowing clear tape. The newer techniques of digital imaging and computer rearrangement of chromosome images are electronic methods for the same task. For publications, photographic images have been thought to give slightly better definition than digital images though with the rapid improvements that have been made in this technology, the differences are no longer very significant. Photographs should be mounted using some kind of adhesive behind the chromosomes so that no details are lost.

The chromosomes were originally categorized by size and morphology into seven groups, A–G (18,19). With the advent of banding, the autosomes (non-sex chromosomes) could also be identified individually: there are 22 pairs of autosomes, numbered 1–22. The sex chromosomes were named X and Y; they were not given numbers but were assigned to groups based on their size. The X chromosome belongs to the C group, and the Y chromosome is part of the G group. When chromosomes are not banded, as in Fig. 1, they can be assigned to groups but cannot all be identified individually. When they are banded by the G, Q, or R banding methods, each chromosome can be positively identified by number as well as by group. The distinguishing characteristics of each chromosome when banded are given later in this chapter.

Principles of Karyotyping

In Fig. 1 the spread from A is arranged as a formal karyotype to illustrate the principles. Submetacentric chromosomes are always arranged with the short arms uppermost, and acrocentrics are placed with the centromere at the top. The groups are arranged in alphabetical order. The sex chromosomes may be placed together at the end or separated to their groups; either way is acceptable.

The largest chromosomes are in the A group (two each of numbers 1, 2, and 3). Chromosomes 1 and 3 are metacentric chromosomes, 1 being larger than 3, and the number 2 chromosomes are slightly submetacentric.

The B group consists of two pairs of chromosomes that are large and strikingly submetacentric; the short arms are about one fourth the length of the long arms. Without banding treatment they cannot be identified individually. They are numbered 4 and 5.

The C group contains the most chromosomes and is the most difficult group to karyotype in both banded and nonbanded spreads. The chromosomes are medium-sized and are all submetacentric; they are numbered from 6 to 12. The X chromosome also belongs to this group. In the nonbanded state, these chromosomes cannot be individually identified, so they are arranged roughly in order of decreasing size: the 6s are the largest, and the 7s and the X are the next largest. Chromosomes X and 7 are about the same size, but the X is more metacentric than the 7. The 11s are the most metacentric, and the 9s are variable in size depending on the length of the heterochromatic region just below the centromere. The 8s, 10s, and 12s have the shorter short arms, and the 12s are the most submetacentric.

The D group consists of three pairs of medium-sized acrocentric chromosomes, numbered 13, 14, and 15. In the nonbanded spread, all Ds look alike. Tiny short arms with satellites may be visible, though they rarely photograph well.

The E group consists of three pairs of chromosomes that are about the same length as the D group chromosomes but have clearly defined short arms. The 16s are metacentric, and the 17s and 18s are submetacentric. The 18s have the shortest short arms in this group.

The F group contains two pairs of small metacentric chromosomes, 19 and 20, that cannot be individually distinguished without banding treatment.

The G group contains two pairs of autosomes, numbers 21 and 22, and the Y sex chromosome. The 21s and 22s are small acrocentrics that frequently have short arms with satellites, similar to the Ds. The Y chromosome can be very similar to the other Gs, although it does not have satellites, and may have more defined short arms. It can also vary in size and is sometimes larger than the 18, although its short arms are always small.

The normal female has two X chromosomes and no Y chromosome, so that the nonbanded spread has 16 C group chromosomes and only 4 G group chromosomes. The normal male, with only one X chromosome, has only 15 C group chromosomes but has 5 G group chromosomes because of the presence of the Y. It is usually fairly simple to count the number of G group chromosomes present and thus determine the sex if the cell is normal.

It is possible to analyze the metaphase spread visually without having it arranged into a formal karyotype by identifying each chromosome by group or by number, either on a photographic print or at the microscope. If the analysis is done with a photograph, letters or numbers can be written directly on the print. If the work is done through the microscope, it is easiest to make notations on a piece of paper indicating the location of each chromosome to determine that the correct numbers of chromosomes are present in each group. Analyzing at the microscope in this fashion sometimes allows an early diagnosis to be made and can save much photographic work. With continued practice, microscopic karyotyping becomes a useful skill.

BANDING AND BANDED KARYOTYPES

Banding is the treatment of a slide to produce bands on the chromosomes (20). A band is defined as that part of a chromosome which is clearly distinguished from its adjacent segments by appearing darker or lighter. When banded, chromosomes appear as a continuous series of dark and light bands, with no unbanded areas. The earliest banding technique used quinacrine fluorescence, but the development of a Giemsa method following enzyme or chemical pretreatment proved to be more acceptable in most parts of the world. The bright bands of the fluorescent quinacrine pattern (Q bands) correspond very closely to the dark bands produced by the Giemsa method (G bands). A second nonfluorescent method, reverse banding (R banding), was developed in France and produces a banding pattern that is almost the opposite of the Giemsa pattern.

The exact mechanism involved in banding is unknown (21,22); the interaction of DNA, associated proteins, and the stain molecules has not been completely elucidated. It is known, however, that the areas that fluoresce brightly with quinacrine or stain darkly with Giemsa have DNA sequences rich in AT pairs. These bands also seem to be the less active portions of the chromosomes in terms of genetic and metabolic impact, and they appear to remain fairly condensed, even in the interphase nucleus. The sections that do not take up these dyes but remain pale appear to be the major areas for active genes. R banding techniques usually involve harsher methods, such as heat denaturation, but, again, the mechanisms are not totally understood. Because the overall distribution of bases is different for each chromosome, each has an individual banding pattern that is recognizable by any of the three methods. After banding treatment, each chromosome can be identified individually, and sections of chromosomes can be studied to find smaller aberrations.

A major difference between banded and nonbanded chromosomes is the loss of the clear two-chromatid structure. This is an artifact of the procedure and does not represent any basic change in structure. The banding treatment (either with fluorescent dyes or with trypsin or heat denaturation) gently swells the two chromatids until they press together and cannot be distinguished from one another.

Karyotyping of banded chromosomes is performed using the same principles described for the nonbanded chromosomes but uses the additional information provided by the banding pattern to identify each chromosome by number. As noted with the nonbanded preparations, repeated practice is recommended, both in cutting and arranging formal karyotypes and in microscopic karyotyping.

At the Paris conference in 1971 (15), a system was devised for identifying whole chromosomes and banded sections numerically; it has been revised to accommodate the greater resolution possible with improved techniques (16,17). A schematic diagram of the banding patterns shown in Fig. 3 is from ISCN 1985 (16); this is unchanged in ISCN 1995 (17) except for band Yq11.22 (dark) which has now been split into Yq11.221 (dark), Yq11.222 (light), and Yq11.223 (dark). Examples of banded karyotypes with Giemsa stain and with quinacrine dye are shown in Figs. 4 and 5.

A more recent development in banding technique is the use of longer chromosomes, in which the wider bands separate into many small bands. High-resolution banding can be obtained by using the standard banding techniques on longer chromosomes. A high yield of prometaphase and prophase chromosomes is produced by treatment of the cultures with chemical inhibitors of chromosome condensation (such as ethidium bromide) or by synchronization of cultures followed by a short period of spindle blocking with colchicine (23–27). Procedures for culturing cells are given elsewhere in this manual.

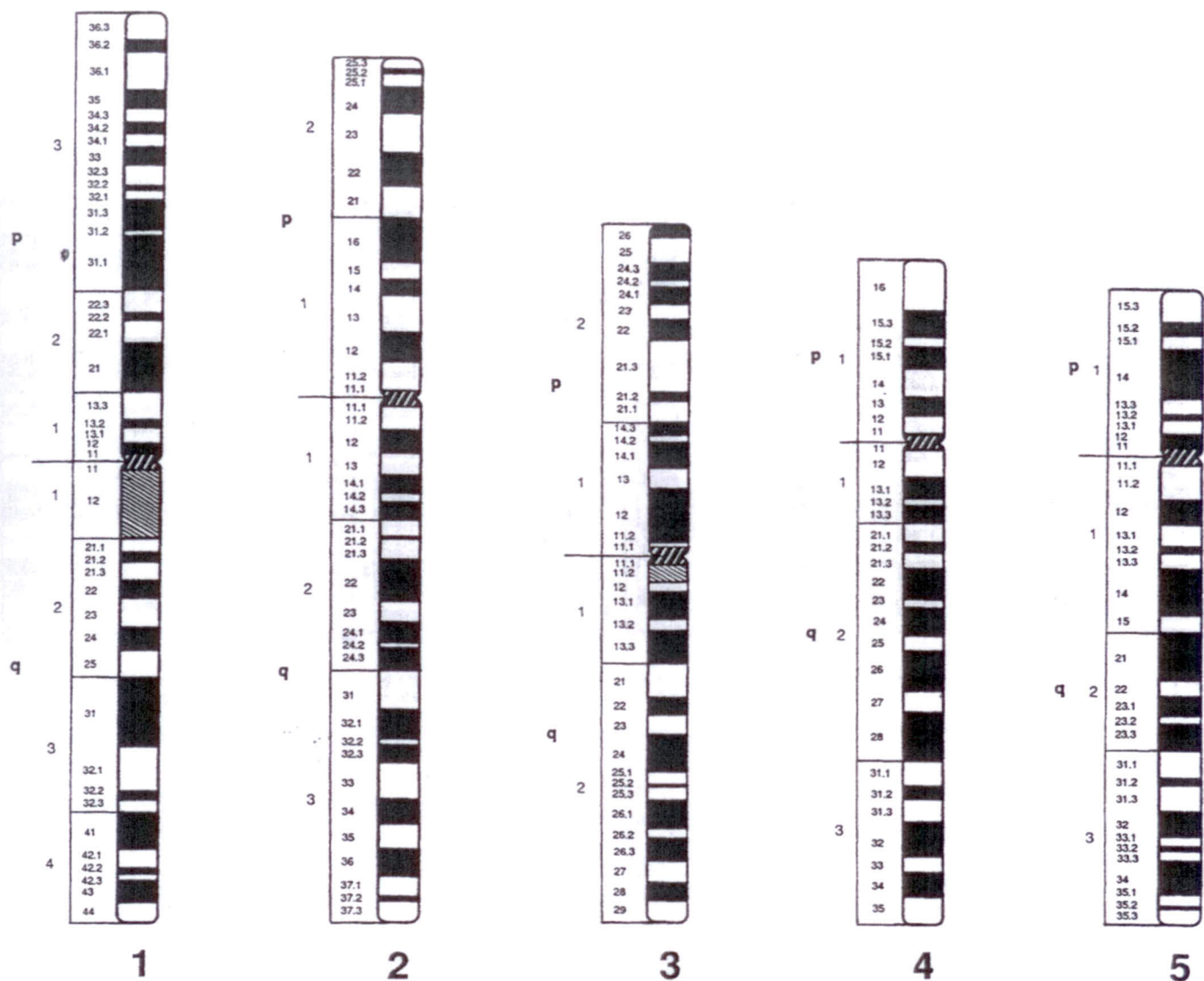


FIG. 3. International schematic for banding pattern adapted from ISCN 1985 (16). The dark bands represent G- and Q- positive bands. The areas with cross-hatching represent variable regions.

Analysis of high-resolution banding is time consuming and may not be practical for routine work. It is a procedure that is extremely useful in looking for breakpoints and in determining the presence or absence of small deletions or insertions. Many clinical syndromes have been shown to be associated with deletions of particular minute chromosome bands. Some of these are discussed in a later section of this chapter. High-resolution banding is recommended for such cases and also for apparently normal adults who have reproductive problems that may be caused by small translocations or deletions. However, even very high-resolution bands can only identify collections of genes, not single genes. For the elucidation of single-gene disorders, the new fluorescent *in situ* hybridization (FISH) techniques are more accurate and much more efficient (28,29). FISH techniques are rec-

ommended whenever deletions within a gene are suspected. As more single-gene defects are identified, more probes are being developed that attach to specific segments of DNA, and deletions are identified by comparison of samples from affected persons with nonaffected controls. Each probe is specific for a particular section of a gene or DNA region; it is essential to have a good clinical diagnosis before beginning FISH procedures to ensure that the testing is focused on the exact area of the genome in question. Another FISH technique, chromosome painting, is used to color whole chromosomes, which can be useful in confirming presence of translocations: two colors of a chromosome-specific probe can be used simultaneously on a slide against a third background color (counterstain) to identify normal and derivative chromosomes (30).



FIG. 3. Continued.

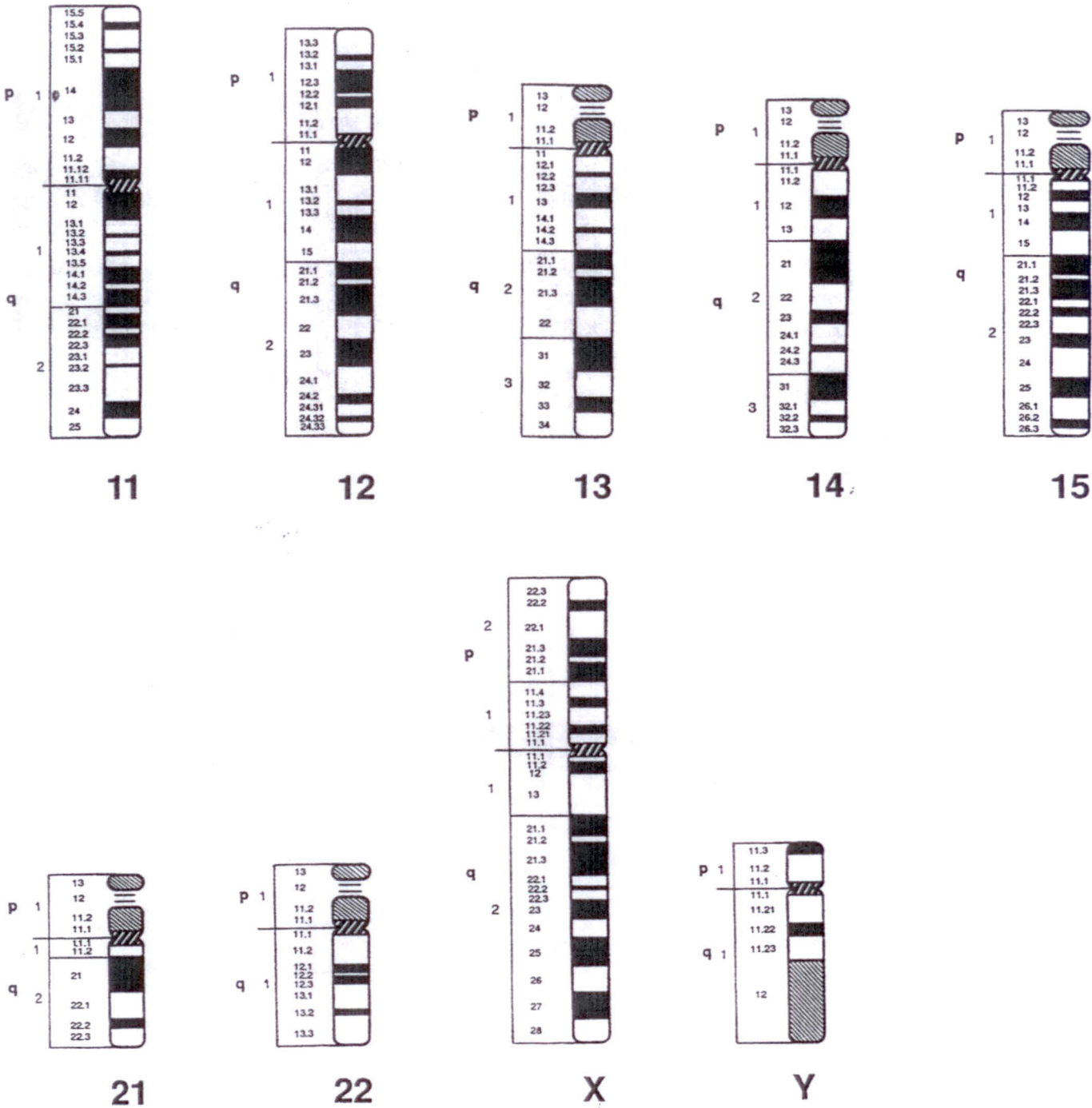


FIG. 3. Continued.

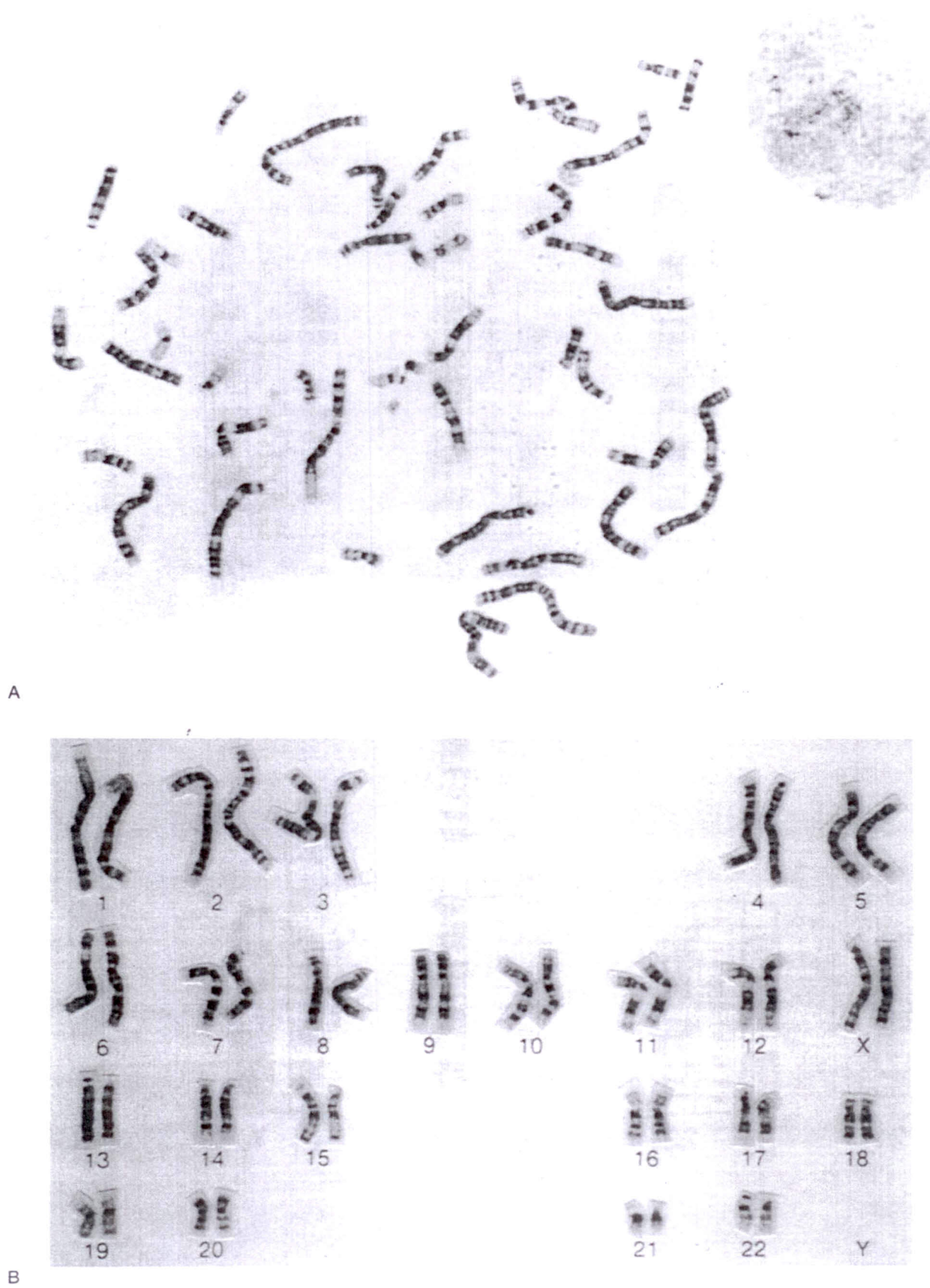


FIG. 4. A: Banded metaphase, GTG. B: 46,XX (normal female karyotype).



FIG. 5. A: Fluorescent-banded metaphase, QFQ. **B:** Karyotype.

Haploid Band Number and Band Levels

Band level is a term representing the total number of bands countable in a haploid set of autosomes plus the sex chromosomes. The band level that can be obtained by the various banding procedures depends on the condensation achieved by the chromosomes as the cell passes through mitosis. It is desirable to aim for as high a level

of band resolution as possible. The landmark bands that were the first demonstrated on metaphase chromosomes represented a band level of about 250. At this stage in late metaphase, all chromosomes can be identified individually, but even quite large abnormalities are invisible. The Paris Conference Schematic (15) represents chromosomes at the 400-band level; this is obtained from chromosomes from a slightly earlier stage of metaphase. It is

the band level adequate for most amniocentesis and peripheral blood samples.

A band level of 550 (see Fig. 3) provides sufficiently high resolution for detection of small rearrangements without at the same time being prohibitively difficult to analyze. This level is obtained from cells at the prophase or prometaphase stages, when several small, distinct bands can be demonstrated that will later condense to form the well-known landmark bands. Some laboratories claim to work with bands at the 850 level or higher; although this might be necessary for some rare cases, it is unsuitable for routine laboratory work. The longer prophase and prometaphase chromosomes are more difficult to interpret but can be mastered with practice (31). A schematic comparison of 550 and 850 level banding and photographic examples of chromosomes with high-resolution banding are given in ISCN 1995 (17). The newest ideograms for chromosomes at the 850-band level in this publication are taken from the work of Francke (32).

It is obviously impossible to count all of the bands in each spread examined, but it is possible to have a few chromosomes to use as guides in estimating the band level of a spread when it is found on the slide and when it is karyotyped. Chromosome 9 is useful in showing the difference between the 250 level and the 400 level. In the short spread, the chromosome appears to have only two large, dark bands in the q arm. At 400-band level, the lower dark band separates into two smaller dark bands separated by a light band. Several other chromosomes are useful in judging whether a spread is at the 400 or 550 level, and these differences are listed in Table 1.

Another more quantitative system for band level assessment has been developed at the Cytogenetics Laboratory of Vancouver General Hospital (33). This system uses the dark Giemsa bands from five different chromosomes, counting all bands except the centromere on the X and 10,

11p, 12q, and 1p31-p32. The total obtained is related to the haploid band levels, from 350 to 850. The number of bands counted represent approximately 7% of the total haploid count at band levels of 500 and above, and slightly less at lower band levels. The sum of the bands on these segments on the ISCN schematics are as follows: 18 bands at the 350-band level, 21 bands at 400, 40 bands at 550, and 60 bands at 850. In counting the actual bands on the chromosomes of a spread, the total obtained might be between the numbers given here, in which case an extrapolation can be made. The system works well because there are clearly developed bands in increasing numbers on the selected chromosomes, as the resolution is improved. It is a relatively quick method that is simple to document in the patient's file. As mentioned in the article, the calculation takes about 2 minutes. Use of this system has been found to increase awareness of band levels and to improve band resolution as the technologists monitor their own work.

Special Banding Techniques and Polymorphisms

Sections on some of the chromosomes are involved neither in producing messenger RNA nor in controlling gene function. Their exact purpose is unknown, but because they are not directly involved in unique cellular functions there appear to be no phenotypic effects if the sections are present in multiple copies. Many of these areas are composed of tightly condensed heterochromatin. In some families, changes have occurred in these regions over many generations, usually by unequal crossing-over in meiosis, which enlarges or reduces them. These normal variants are useful in studying inheritance patterns in some families (34–36). Some heterochromatic regions of the chromosomes are strongly stained by both fluorescent banding (Q banding) and heterochromatin banding (C banding) (37). Descriptions of these polymorphisms are given in the descriptions of the individual chromosomes.

Other stains are useful under some special circumstances. The most widely used special stain is silver nitrate staining, which identifies the NORs on the short arms of the D and G group chromosomes. It is particularly useful in identifying the origin of small additional fragments and in tracking familial polymorphisms of the acrocentric chromosomes (38).

THE COMPLETE CYTOGENETIC STUDY

The American College of Medical Genetics (ACMG) has published Standards and Guidelines concerning the components of the complete cytogenetic study, which are followed by most laboratories (2).

These recommendations are similar to those used for the annual laboratory inspections by the College of American Pathologists (CAP) and others published by the

TABLE 1. Comparison of bands seen on four chromosomes at two different levels of band separation

Chromosome	Approx. 400	550–600
7q	2 dark bands	Each splits to 2 dark and 1 light
10q	3 dark bands (top one is darkest)	Top dark band splits to 2 dark and 1 light; also 1 dark band appears in next distal light band, q22
11p	2 dark bands, very close together	2 dark bands separate and a thin dark band appears, close to terminal
Xp	1 large dark band	Splits to 2 dark & 1 light between; upper dark band is largest

Data from ISCN 1995 (17).

Association of Cytogenetic Technologists (39). (The ACT guidelines are reprinted in Chapter 15.) Each patient sample is assigned an identifying number when it is received at the laboratory. Unless the sample is too small for more than one culture, multiple cultures are usually initiated and processed through the harvesting stage. All cultures and slides must be labeled with the identifying number and the patient's name. Because several cells must be counted and analyzed to determine the modal number (the number of chromosomes per cell occurring most frequently in the sample), several slides will be needed. The number of counts that must be performed depends on the nature of the study and on the type of specimen involved. Some form of tally sheet is required for documentation that the study has been conducted properly. It must be organized so that it has space for adequate patient and sample identification and for all notes concerning the reading of the slide and the preparation of karyotypes. Each laboratory will have a particular format for this tally sheet, with spaces for vernier locations and any relevant comments such as abnormal morphology, abnormal numbers, breaks in chromosomes, and number of photographs taken. It should also give the identity of the person who performed the analysis.

Each slide must be examined in an orderly manner. The slides should always be placed on the microscope stage with the label at the same end and scanned in overlapping rows so that the entire slide is examined. Vertical scanning is generally preferred because horizontal scanning may cause nausea in persons susceptible to motion sickness. Scanning is done at low magnification (10x objective). When a spread is found that might be suitable for counting, the objective is changed to the high dry or oil objective (60x or 100x) for a more detailed examination. Any suitable spread found should be counted to determine that all groups of chromosomes are correctly represented. The location of the spread on the slide, according to the microscope vernier readings, should be noted on a tally sheet, and some indication of the quality of the spread should be given also. A spread is a good candidate for counting and analysis if it is well separated from all other spreads and if all the chromosomes can be clearly identified with good morphology and, if possible, no overlaps.

For many samples, the quality of the spreads chosen for analysis should be the best that can be found on the slide. The major exception to this rule is in cancer cytogenetics, in which the abnormal cells are sometimes of poorer quality than the normal cells; this point is discussed more fully in the chapters on cancer cytogenetics. For mosaic specimens, consecutive counts are important when obtaining the ratio of different cell lines; this may lead to analysis of spreads of lesser quality.

It is good practice to rate the quality of the spreads: excellent for spreads with sharp, clear bands and no overlaps; good for spreads in which all the chromosomes can be identified, though some overlaps are present; and fair

for spreads that can be analyzed but are unsuitable for photography and karyotyping.

Mosaicism

The number of spreads counted must be sufficient to determine the modal number and to eliminate the possibility of significant mosaicism. Mosaicism is the presence of two or more chromosomally distinct cell lines, each seen in two or more cells. It arises after fertilization through inaccurate segregation of chromosomes at mitosis. Two mechanisms have been postulated to account for errors of segregation, as shown in Fig. 6. Nondisjunction is the failure of the two chromatids to separate at the centromere at metaphase, causing both chromatids to go to the same daughter cell and leading to one hyperdiploid (more than the correct diploid number) cell and one hypodiploid (less than the correct diploid number) cell. Anaphase lag is the loss of a chromatid at anaphase because it is not attached to the spindle and so does not get drawn to the pole. This causes one hypodiploid cell, though the other daughter cell of the pair can be normal (40-44). Sometimes, if a balanced translocation is present in a patient, there is a problem forming meiotic figures and in preparing for disjunction. A condition called 3:1 meiotic disjunction can occur, with unequal sharing of the translocation chromosomes as the cell divides. A report of a child with two aberrant chromosome 7s inherited from the mother and no paternal chromosome 7 (uniparental disomy) has led to speculation about other aberrant cell division mechanisms; fortunately, these aberrations occur extremely rarely (45).

The level of mosaicism depends on the stage of development of the organism when the error in division occurs (Fig. 7). If misdivision occurs at the first cell division after fertilization, it is possible for all tissues in the body to be affected, with mosaic lines of 50% each. If the misdivision occurs after the three cell types (ectoderm, mesoderm, and endoderm) have been established, the abnormal cells could be localized to one cell type; if it occurs later still, abnormalities could appear in only one organ of the body. The level of abnormal effect could be dependent on the percentage and location of abnormal cells present in the body.

The cells of the body are related to each other through a continuous branching of the developmental tree, rather like a relationship in a family tree. The initial development is the growth from a single cell to a small ball of many cells (a blastocyst), and the secondary development is the development of the ectoderm, mesoderm, and endoderm cell types. The organs of the body develop from these embryonic cell types, as shown in Fig. 8. It might be necessary to examine tissues from a different cell type if mosaicism is suspected but not apparent in the tissue sample submitted.

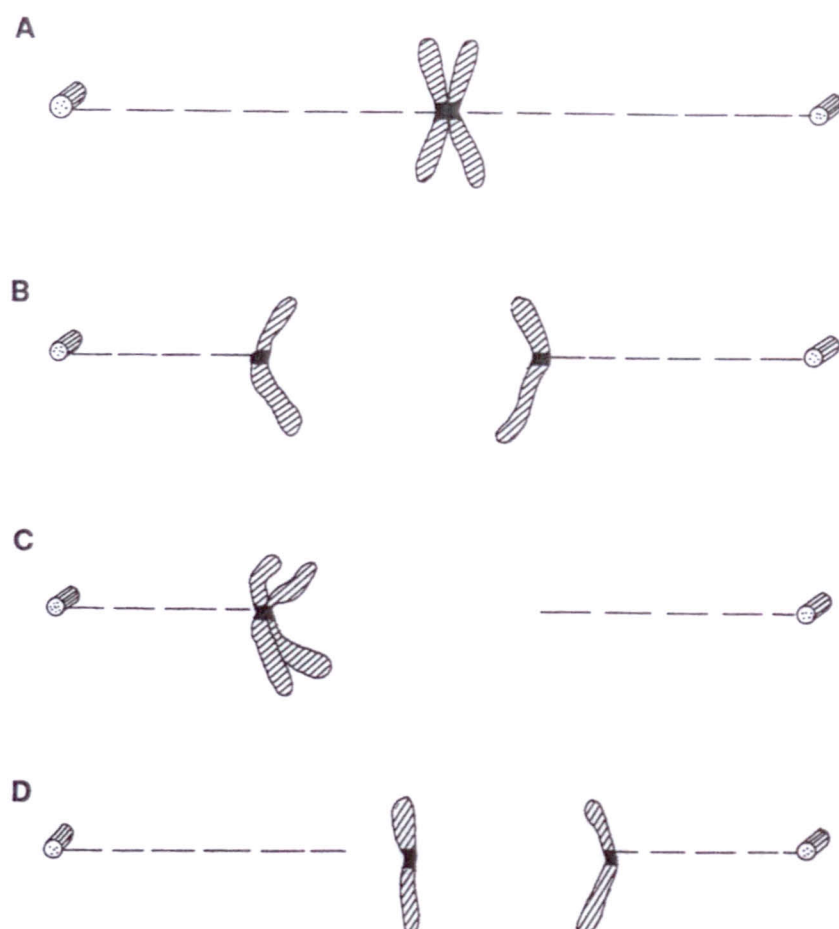


FIG. 6. Normal and abnormal chromatid separation at mitosis. **A:** Chromosomes at metaphase plate between centrioles. **B:** Normal disjunction at anaphase—one chromosome to each centriole. **C:** Nondisjunction at anaphase—two chromatids to one centriole. **D:** Anaphase lag—one chromatid fails to attach to spindle.

Mosaicism must also be considered as a result of the impact of viruses and chemicals on dividing cells during life. In the cytogenetic analysis of oncology samples, mosaicism is a constant factor under consideration (see Chapters 7 and 8).

In prenatal diagnosis, each cell line must be observed in more than one independent culture. There is no way to totally eliminate the possibility of mosaicism in the patient

under study because the sample does not contain all of the patient's cells. The sample is representative of the cells of the body from which it came, however, so that it is possible to count enough cells to eliminate certain levels of mosaicism. Statistical tables have been developed to indicate what level of mosaicism is ruled out by counting certain numbers of cells. Hook's tables (46) apply to lymphocyte studies, and the studies of Clausen (47) and Cheung

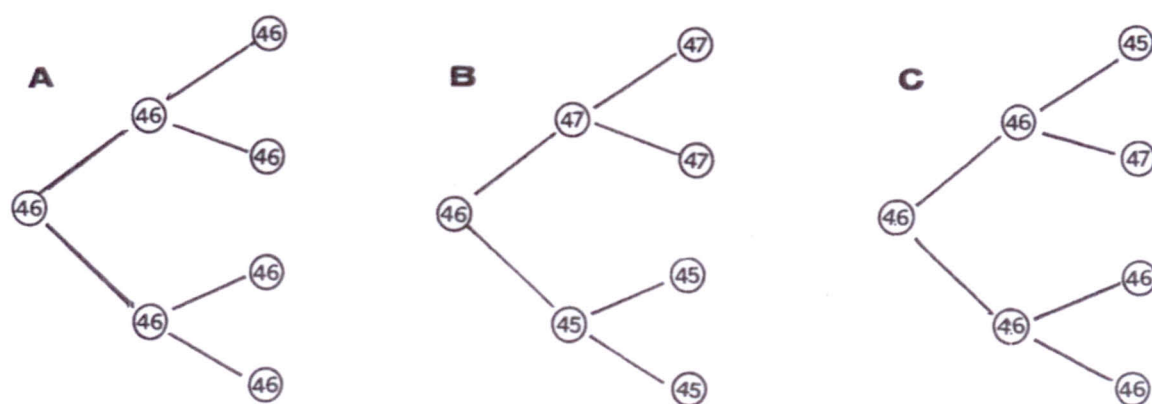


FIG. 7. The development of mosaicism. **A:** Normal cell division. **B:** Unbalanced segregation at the first mitosis. **C:** Unbalanced segregation at the second mitosis.

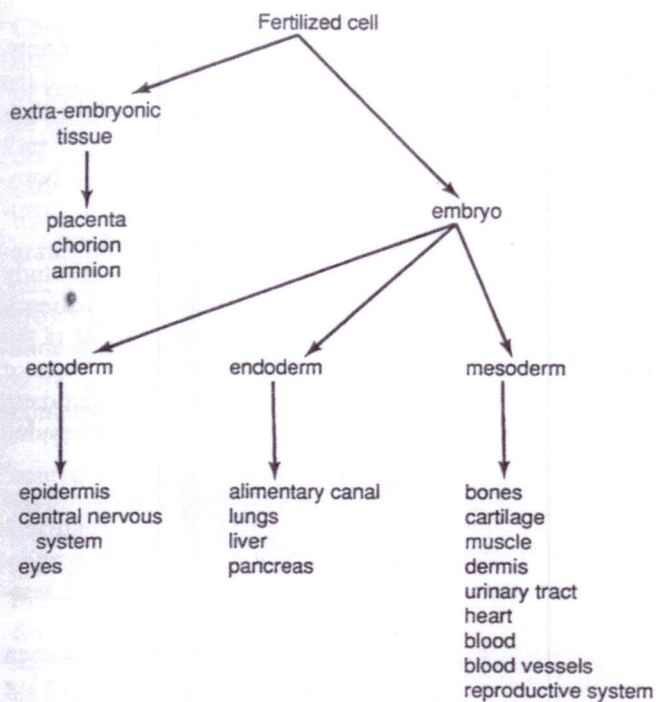


FIG. 8. The genealogy of the organs of the body. In checking for mosaicism, tissue from different groups should be examined. Blood is mesodermal in origin, skin is ectodermal.

(48) are calculated for amniotic fluids. In each study, the statistical analysis is based on the assumption that the sample obtained is truly random (though it probably is not) and that all cells grow at the same rate and behave the same in the *in vitro* culture flask (which is probably not true either). However, these tables are very useful as a guideline for assessing the confidence levels for mosaicism because they show the percentage of mosaicism excluded and the confidence levels that may be accepted if a specific number of cells have identical counts. The recommended count of 20 identical cells on a patient means that mosaicism of greater than 14% may be excluded with a confidence level of 95%. Ruling out mosaicism of greater than 10% for the same confidence level requires counting 30 identical cells on a sample. Counting 50 identical cells rules out only mosaicism of greater than 6%, with the same confidence level. The counting work involved becomes more than the circumstances warrant unless there is good reason to suspect from the clinical diagnosis that low-level mosaicism may be present. For most constitutional studies, counting 20 cells and finding them identical is sufficient to provide the cytogenetic diagnosis. It may be necessary to examine a greater number of cells or to obtain a sample from a different tissue source when the clinical diagnosis strongly suggests a syndrome that is not found by studying 20 metaphases from a lymphocyte culture. For some cancer work or for investigation of fragile sites or breakage syndromes, it may be necessary to analyze even greater numbers of cells.

In counting 20 cells, if one cell is found with a different karyotype that may represent a clone of different cells, additional counting is indicated, usually to 30 cells. With amniotic fluid or lymphocyte cultures, if no further cells are found identical to the one different cell already noted, then the aberrant cell can be assumed to be artifactual, and mosaicism of greater than 10% can be excluded at a 95% confidence level. If other aberrant cells are found, low-level mosaicism may be present and should be reported, particularly if the karyotype of the minor line represents a known syndrome. Mosaicism for chromosomes 2 and 16 is sometimes found as an artifact of amniotic fluid cultures and usually has no clinical significance. With bone marrow samples, even a single aberrant cell might represent a neoplastic line and may need to be reported (see ISCN). Because it is possible for aberrant cells to arise from the long-term culturing process necessary for amniotic fluids and tissues, several criteria should be met in order to differentiate true mosaicism from sporadic artifactual changes (pseudomosaicism). These have been discussed extensively in the literature (41,42,47-58).

1. Nonmodal chromosome counts must be too frequent to be attributed to chance alone.
2. The nonmodal cells must have a constant number of chromosomes and the same karyotype. Particular care must be taken if the karyotype represents a known syndrome.
3. The bimodal count must be present in more than one culture flask and must be reproducible in successive cultures of the sample. For this reason it is wise to consider requesting a second sample to count more than 50 cells if mosaicism is suspected and if a repeat sample can be obtained with relative ease. It may also be necessary to consider other methods of obtaining confirmatory information, such as DNA probes.

Because artifactual changes are known to occur during the tissue culture process and because fetal cells may be more readily susceptible to external influences, it is necessary to be certain of counting cells from several different clones when working with amniotic fluid cells. This means counting cells from more than one culture dish, even if using the *in situ* method with several clones on a single coverslip. Counting from more than one culture dish acts as a safeguard against reporting a false karyotype when maternal cell contamination is present and also helps to differentiate true mosaicism from pseudomosaicism.

If mosaicism is suspected in cells derived from amniotic fluid culture, it becomes essential to determine if this mosaicism reflects the actual state of the fetus. If a single colony exhibits mosaicism, count or score (scan spreads for particular chromosomes) an additional 10-20 cells: How many colonies in the culture vessel are affected? How many culture vessels are affected?

It is not possible or necessary to determine how many cells in the aberrant clone are affected, although it may be possible with *in situ* preparations to show that more than one cell line is present within a single clone. It is usual to assume that each clone starts from a single cell, but in practice, cells frequently attach very closely together, and colonies can have a multicell origin. All clones on the slide must be examined to determine if any others are affected, and it is necessary to count or score from any additional cultures that are available. If the aberrant cell line is confined to a single colony or to one culture vessel, the aberration probably represents an artifact of culture (pseudomosaicism); if it can be demonstrated in more than one culture vessel, however, it probably represents true mosaicism.

With flask cultures, it is impossible to guess how many colonies in the culture vessel are affected. However, the number of culture vessels affected is the more important question, and flask cultures can be as helpful in answering this as *in situ* cultures. The additional cells counted or scored should come from an independent culture if possible.

Chorionic villus samples are more likely to have aberrant cells than amniotic fluids because the sample is from the placenta, not from the fetus itself: more variations of chromosome constitution seem to be permitted in the extraembryonic tissues (59). Any mosaicism observed either in direct analysis or after culturing of cells should be considered an ambiguous result requiring follow-up by amniocentesis or fetal blood sampling.

Mosaicism for an extra whole chromosome in liveborn infants has so far been reported for the following chromosomes, in order of greatest to least incidence: 21, X, 18, 13, Y, 8, 9, 14, 22, 20, 12, 10, and 7. Such mosaicism is extremely rare, but greater care is required when considering the importance of a cell having one of these chromosomes as the extra chromosome. Only the X and 21 chromosomes have been reported in liveborn infants as mosaic monosomies. Each laboratory must have written guidelines for procedures to follow for assessing general types of abnormalities (aneuploidy and structural changes) and for some specific abnormalities (e.g., -2, -16, -20, loss of a sex chromosome) (39,41).

Photographs, Karyotypes, and the Final Report

After a case has been analyzed microscopically, images are made of representative metaphase cells either with a photographic or digital system. The number of images to be made will be established by the laboratory director. With fluorescent stain, it may be necessary to make prints of many more cells because it is easier to analyze prints than to analyze through the microscope; prints enable technologists to check their analyses. Some laboratories, particularly those in cancer work, photograph as many as 30 consecutive spreads for later analysis.

All photographic materials, both negatives and prints, must be clearly identified with the patient's name and the case number, and careful records must be kept of the photographic work, either in a special file or in each patient's file, following the protocols established for each laboratory. A system of identification of digital images is usually programmed into the computer imaging system.

The final report must be prepared as soon as the study is completed, and a copy should be sent to the patient's physician and to the medical records department if the patient is admitted to a hospital. Four major categories of information are relevant to the final report: patient data; specimen data; laboratory data; and cytogenetic results, interpretation, and recommendations.

Patient data include the name and address of the patient, the patient's age or date of birth, any identification number, the phenotypic sex of the patient, the referring physician, clinical indications for cytogenetic testing, and relevant family history.

Specimen data include the source of the specimen (amniotic fluid, blood, etc.), the date and time of drawing from the patient and reception at the laboratory, and notes on any unusual qualities of the specimen at receipt, during culture, and at harvest that may affect the outcome of the study.

Laboratory data should state the number of cultures set up, the number of cells counted and analyzed, the karyotypes, any variants noted, any unusual finding such as breaks or gaps, the type of stains used, and the overall quality of the preparations.

The cytogenetic results, interpretation, and recommendations should also be listed on the final report. A statement of the patient's cytogenetic findings using ISCN 1995 (17) and an interpretation of the scientific nomenclature in terms that the physician will understand must be included. The report should also contain comments on the correlation between the cytogenetic findings and the patient's clinical presentation and any recommendations for counseling or further cytogenetic studies of the patient or of the immediate family.

Some final reports include copies of the karyotypes, but because of the time and expense of producing these copies, most laboratories no longer include them unless there is some abnormality to report.

Sources of Error in Analysis and Reporting

It is extremely important to avoid errors in genetic studies, especially with cases involving prenatal cytogenetic diagnoses or with neonates requiring life support measures (60). Because almost all stages of a cytogenetic study involve subjectivity and individual expertise on the part of the technologist, it is important to be aware of some potential sources of errors.

Mistakes can occur at various stages, from specimen collection to the actual interpretation of the karyotype.

Cases can be mixed up during culturing, harvesting, slide making, slide or record sheet selection at the microscope, or printing of the film and karyotyping. Each case must be properly labeled throughout the entire cytogenetic procedure—preferably by the patient's name and an identification number. It is also helpful to work in an atmosphere that is free from distractions and to handle each case separately when setting up cultures, making slides, printing, and karyotyping. Extreme care should be taken to avoid cross-contamination of specimens during harvesting and slide making. It may sometimes be necessary to repeat work using primary cultures (for example, if maternal contamination is found).

Poor growth of cultures and poor banding also present sources of potential error to the technologist. Cultures that yield few metaphases of poor quality—such as compact spreads and cells with many overlapping chromosomes or background debris—may be responsible for incorrect microscopic analysis because the technologist may fail to detect mosaicism, translocations, ring or marker chromosomes, or other minute chromosome aberrations. Poor-quality banding may also be a source of error by preventing the detection of translocations, duplications, deletions, or other structural abnormalities. If the technologist has any doubt, new slides should be prepared, a harvest repeated on a culture, or a repeat specimen requested.

Extreme caution should be used to avoid making subjective interpretations of the finished product—the karyotype. The banding pattern of each chromosome should be closely examined in each study, and every chromosome pair should be compared band for band at least once to ensure that no differences exist between homologous chromosomes. If an unusual pattern is observed, all means necessary, including supplementary banding or molecular procedures, should be employed to rule out or confirm any abnormality. More than one karyotype must be prepared, and further examination of questionable chromosomes must be made, either microscopically or with printed examples. Sometimes a weird chromosome in a printed karyotype can be due to variations in the slide making and staining, limitations of the image reproduction system, or to the location of the chromosome within the metaphase: chromosomes tend to stretch on the outer edge of the metaphase, overlapping chromosomes can confuse banding patterns, and extraneous stain can be converted to very realistic chromosome segments.

Finally, obtaining another opinion is valuable. Even if a technologist works alone, consultation with other laboratories is easily accomplished by telephone and by mail and may prove invaluable in many cases.

Storage of Records

The length of time that records must be kept is usually mandated by the various regulatory agencies that oversee the quality of the laboratories. Each state has its own par-

ticular requirements, and each laboratory will have to comply with those requirements. Slides do not remain readable for many years; the stains are somewhat unstable and fade. Photographic negatives can be stored indefinitely, however, and new prints can be made at any time. The newer digital image systems usually have a method for archiving records. It is not yet clear if these methods are as stable as photographic negatives. Storage of patient information on paper or in computer record systems makes the information available for many years, though reasonable care must be taken to protect the records from heat and moisture. The statute of limitations for litigation is 7 years after reaching majority. In some cases, majority is not reached for 18 years, and some records need to be available for 25 years. The storage space required for 25 years of laboratory records is quite large, and methods for condensing records could be important.

ABNORMALITIES INVOLVING WHOLE CHROMOSOMES

Polyploidy

The human genetic complement is normally composed of 22 autosomes and two sex chromosomes, and this is known as the diploid state ($2n$). It is formed from the union of two haploid cells at fertilization (each having 1n chromosomes). Cell lines containing multiples of the haploid number other than diploid—such as triploid ($3n$) and tetraploid ($4n$)—are called polyploid. Triploid karyotypes have been reported both in spontaneous abortions and in full-term live births. The abnormal cell constitution can be due to fertilization of an ovum by two sperm or fertilization of an ovum that has not discarded the final polar body. Reports have included three sexual types: 69,XXX; 69,XXY; and 69,XYY. Tetraploidy (92 chromosomes) is a common artifact of tissue culture. Cases have been reported of triploid and tetraploid newborns and amniotic fluid samples. True tetraploid cases are fortunately rare. A culture that proved to have only tetraploid cells would be highly unusual, and the laboratory might then call for additional study of the fetus by repetition of the amniotic fluid analysis with a fresh sample, careful ultrasound analysis, or both. All liveborn nonmosaic polyploid infants have died within a few hours of birth (61).

Endoreduplication is a term applied to a special type of polyploidy seen in tissue culture, which arises from failure of the centromeres to separate during anaphase. The attached chromatids undergo DNA synthesis a second time, appearing at the next metaphase as four chromatids fastened together at the centromere. Endoreduplication probably occurs rarely *in vivo*. Because it has been seen occasionally in direct harvest from a bone marrow sample, endoreduplication may be a possible variation in tumor cells, which frequently evolve to a polyploid status.

Variations from the normal diploid state that involve addition or subtraction of single chromosomes, rather than of haploid sets, are aneuploid. Large variations from the normal pattern lead to nonviable fetuses that usually abort in the first trimester. Small variations from the normal pattern can lead to the birth of children with varying types of birth defects depending on the nature of the chromosomes involved in the imbalance. Brief descriptions and illustrations of karyotypes for the most common constitutional aneuploidies are given in the following sections. Note that although syndromes were originally designated by the name of the person who first described them (and these names are included for completeness), it is now preferable to refer to the syndromes by the name of the abnormal chromosome (62).

Trisomy 21 (Down Syndrome)

This is the most common chromosomal aberration leading to live birth (Fig. 9). In 92.5% of the cases, the additional chromosome 21 is present because of nondisjunction in meiosis, and about 25% of these nondisjunction cases can be traced to paternal origin. The other 75% of these cases have maternal origin, and about 50% are linked to advanced maternal age. Paternal age has not been shown to have any relevance. Of the remaining 7.5% of cases, 4.8% are due to translocations that are either inherited from a carrier parent or *de novo*; the remaining 2.7% are caused by mosaicism developing in early embryogenesis (63).

The overall incidence of trisomy 21 is about 1 per 700 live births, with a slightly higher ratio of males to females (3:2). Analysis of the data shows that trisomy 21 occurs in about 1 per 2000 of the births to mothers aged 18–24. The rate gradually increases in frequency to 1 per 300 births to mothers between 25 and 35 and then increases to 1 per 100 births to mothers at age 40 and 1 per 50 births to mothers at age 45 or older (64,65). In the past, life

expectancy for these children was short because of a tendency to succumb to respiratory infections. Because better medical care is now available, more children survive with trisomy 21. Approximately 8% live past 40, and 2.6% live past 50.

Mental ability is limited in these patients, who have an IQ in the range of 25–70. With early intervention, some can be trained to be somewhat independent and even to be responsible enough for simple repetitive employment. Patients usually age prematurely and may have psychotic disorders in later life. Recently, an increased tendency to senility similar to Alzheimer’s disease has been reported. After childhood, the tendency to suffer respiratory disorders decreases, but cardiac and digestive malformations may bring complications. There is a 10-fold increased risk of cancer, particularly acute lymphocytic and nonlymphocytic leukemia, in trisomy 21 patients, and they are less likely to respond to treatment (66).

Females with trisomy 21 are fertile and have had children; the children can be normal or can have trisomy 21. Males with trisomy 21 can be fertile, but their libido is poor. There is no record of children born to a male with trisomy 21.

Trisomy 18 (Edwards’ Syndrome)

This autosomal trisomy has a frequency of approximately 1 per 6000 live births (Fig. 10). It is probably caused by primary meiotic nondisjunction and may be affected by advanced maternal age. The defects observed in children with trisomy 18 are more severe than those seen with trisomy 21, possibly because the amount of genetic material involved is so much greater. Patients usually suffer severe malformations of the heart, kidney, and digestive system along with the more obvious skeletal malformations of rocker-bottom feet and tightly clenched hands. Mental retardation is also severe.

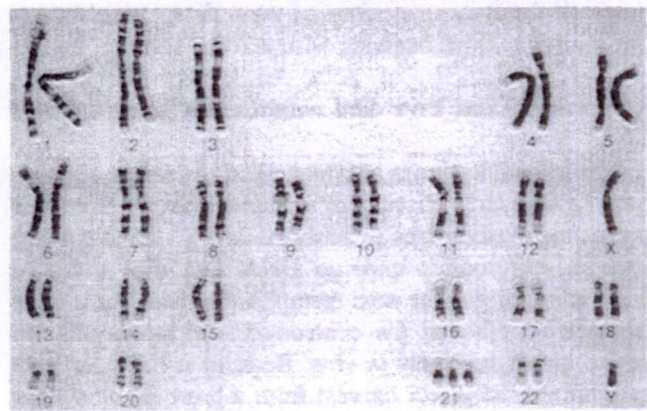


FIG. 9. Trisomy 21 (Down syndrome) karyotype (47,XY,+21).

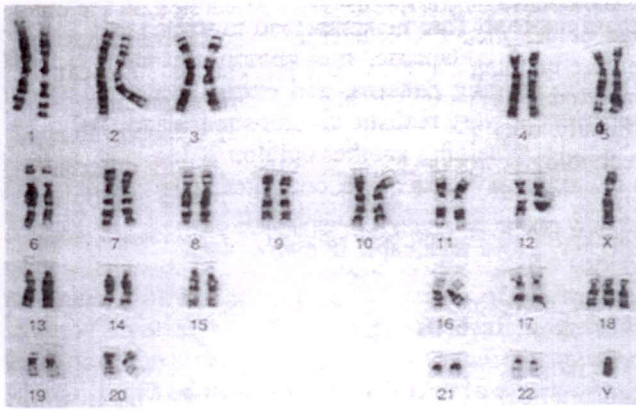


FIG. 10. Trisomy 18 karyotype (47,XY,+18).

Generally the babies are not premature, but birth weight is low—usually less than 2300 g. There is an increased incidence of females over males (4:1). Life expectancy is much shorter than for trisomy 21 infants; 30% of the children die in the first month, and 50% by the end of the second month. Only 10% live beyond their first birthday, but of these, some have survived to the teen years (67). There has been a recent report of a patient with mosaic trisomy 18 without mental retardation (68).

Trisomy 13 (Patau's Syndrome)

This autosomal trisomy has a lower frequency than trisomy 18 (about 1 per 8000–10,000 thousand) and has more severe effects (Fig. 11). The most obvious problems are the midline defects, such as cleft lip and palate. Heart defects are common, as are kidney problems and digestive tract anomalies. Mental retardation is severe. Because of the severity of the defects, 45% die within the first month, with most of them barely surviving birth. Only 5% survive for more than 3 years; there is one report of a patient more than 30 years old (67).

Other Autosomal Aberrations

Other autosomes that have so far been reported as homogeneous trisomies in live births include chromosomes 7, 9, 10, 12, and 22. The number of cases reported is extremely small and the abnormalities related to the presence of the extra chromosome are sufficiently severe to lead to early demise in most cases (69). All of the trisomies mentioned above also occur in mosaic form. Because of the presence of some normal cells the effects of a mosaic trisomy may be less severe than those of a homogeneous trisomy. For this same reason, some trisomies can occur in mosaic form that would be lethal in homogeneous form. The most common of these is trisomy 8 (Warkany's syndrome). A review of the literature

(70) includes four cases that were reported as homogeneous trisomies, but it is felt that perhaps insufficient effort was made to exclude mosaicism in these cases. Other whole chromosomes that have been reported to occur as mosaic trisomies are 12, 14, 20, and 22. These cases are very rare, and the level of mosaicism is always very low (less than 10%).

There are as yet no confirmed reports of homogeneous autosomal monosomies. It is reasonable to assume that loss of a whole autosome is lethal. A report of monosomy 21 was later discovered to be due to a translocation $t(5;21)$, with loss of 5p (71). Monosomy in mosaic form has been reported only for the smallest autosome, chromosome 21, and the cases are very few in number.

Variation in Sex Chromosome Complement

Many variations of sex chromosome complement are compatible with life and are among the most common chromosome abnormalities seen. Each condition has its own set of physical characteristics but premature gonadal failure appears to be common to all (72), except 47,XXX females and 47,XYY males.

Monosomy X (Turner's Syndrome)

The most common sex chromosome abnormality is monosomy X (45,X), with an incidence of 1 per 700 live female births (Fig. 12). The lost chromosome might have been either an X or a Y, but the absence of the second sex chromosome produces a phenotypic female with short stature and various physical abnormalities that are not usually very severe. Mental retardation is not a necessary effect of monosomy X, and with proper medical care, including hormone treatments, Turner's syndrome patients can have a reasonably normal life. They are usually unable to have children; patients reported in the literature to have had children were probably mosaics.

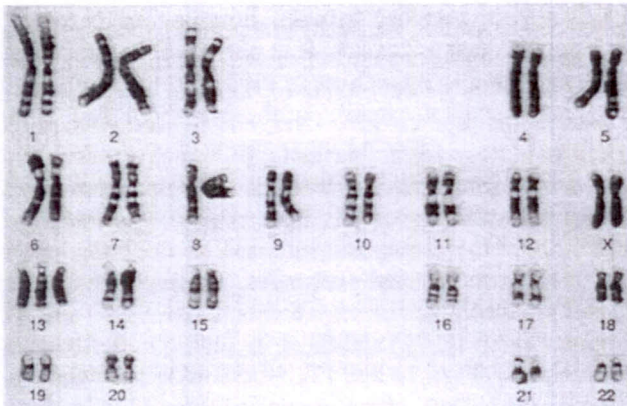


FIG. 11. Trisomy 13 karyotype (47,XX,+13).



FIG. 12. Monosomy X (Turner's syndrome) karyotype (45,X).

Monosomy X is frequently detected at birth because of the characteristic lymphedema of the hands and feet in the affected newborn. With the increased use of ultrasound for fetal monitoring, cases of monosomy X have been detected *in utero* by the noting of unusual lymphedema in the neck region. The heart defect that frequently occurs in these patients, coarctation of the aorta, may also be detected at birth; webbing of the neck may not be obvious, though there may be laxity of the neck skin (73).

There are variant forms of Turner's syndrome brought about by the inheritance of one structurally abnormal X chromosome, such as an isochromosome or a deleted form. These cases are discussed later in the chapter. They are less common than simple monosomy, though they have many features in common with it. Cases of Turner's syndrome with a deleted Y chromosome have also been reported (74).

Turner's syndrome also occurs in mosaic form, associated with either a normal male or a normal female cell line, and with Klinefelter cells or triple X (75–78). Because this can occur, a result of 45,X from an amniotic fluid test should be followed by careful ultrasound examination particularly of the genital region.

47,XXY (Klinefelter's Syndrome)

This karyotype occurs in about 2 per 1000 live male births but is not usually detected until the patient reaches puberty (Fig. 13). The main features are increased height, slightly feminine hip formation and stance, small penis and testes, and possible gynecomastia. There may be mild mental retardation, and some psychological problems may occur. The patient is usually sterile (79).

Variations of this syndrome occur with the presence of more than two X chromosomes. Usually mental retardation becomes increasingly severe with the two known forms: 48,XXXY and 49,XXXXY. The karyotype also

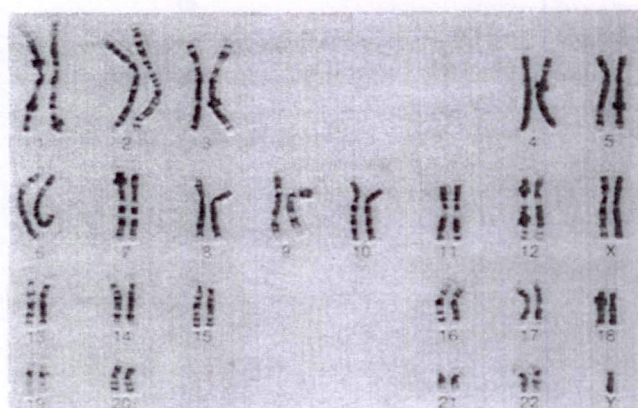


FIG. 13. XXY (Klinefelter's syndrome) karyotype (47,XXY).

occurs in mosaic form with either a normal male-associated or a normal female-associated cell line. The karyotype of an individual with XXY and trisomy 18 is given in Fig. 14.

47,XXX Female and 47,XYY Male

These two forms of the human chromosome complement have very few phenotypic manifestations, and most of the persons with these karyotypes lead normal lives. The aberrations may not be detected unless some other unrelated abnormality is discovered in the family. Both types are fertile and usually produce normal children, though there may be a slightly increased risk of meiotic nondisjunction in triple-X females (Fig. 15), which leads to birth of children with other trisomies (e.g., trisomy 21). A decrease in intelligence frequently occurs, though it is usually not sufficiently severe to be classified as retardation. XYY males (Fig. 16) are usually well above average height as adults. Early reports that XYY males are more likely to have excessively violent natures have been discredited by later studies.

STRUCTURAL ABNORMALITIES CAUSED BY CHROMOSOMAL BREAKAGE AND REARRANGEMENT

Chromosome breakage is not a rare event. All cells have enzymes to repair broken DNA strands, and such repair goes on throughout the life of each cell. It is now known that there are preferred sites for chromosome breakage and that these sites have a high correspondence with known fragile sites (80). During mitosis, when chromosomes have replicated and are waiting to separate into two daughter cells, chromosome breakage is frequently accompanied by exchange of material from one chromatid to another. Such exchange, termed sister chromatid exchange (SCE), is not visible without special staining and usually does not lead to any detectable effects.

Exchange of material between homologous chromosomes occurs during the pachytene period of the first meiotic division and is a mechanism built into the reproductive cycle to ensure the mixing of the gene pool (44). This exchange is called crossing over, and it occurs when the paired homologs form chiasmata. SCE and crossing over are normal occurrences in somatic cells and germ cells, respectively, and are usually undetectable. Abnormalities arise only if the chromosomes break at nonhomologous sites leading to unequal exchanges. There are two major classes of detectable rearrangements: those within a single chromosome (Fig. 17) and those involving more than one chromosome (Figs. 18 and 19). Borgaonkar has published a large catalog of cases resulting from chromosomal rearrangements (81).



FIG. 14. 48,XXY,+18 karyotype.

Rearrangements within a Single Chromosome

Deletion

Deletion is a loss of chromosomal material. An interstitial deletion can arise from unequal crossing over at meiosis; if breaks occur in two homologs at slightly different positions on the chromosomes and if the pieces are reconnected to the alternate chromosome, one chromosome could lose material, and the other one could gain. This mechanism is perhaps more likely to occur than multiple breaks in a single chromosome (82). Two breaks within the same arm of the chromosome could give rise

to three pieces of chromosome: an acentric fragment that becomes lost and the distal and proximal sections that rejoin to form a shortened chromosome. Because the chromosome needs to have a terminal cap attached, a true terminal deletion is unlikely to occur.

Various syndromes resulting from deletions have been described. The most well known is cri-du-chat syndrome, deletion of the short arm of chromosome 5 from band p12 (the size of the deleted fragment varies) to the terminal cap. Some details of this and other deletion syndromes are given in later sections of this chapter. Many deletions are *de novo* (83), with a very few exceptions (84,85). Note that many deletions now being found with FISH tech-



FIG. 15. Triple-X karyotype (47,XXX).



FIG. 16. XYY karyotype (47,XYY).

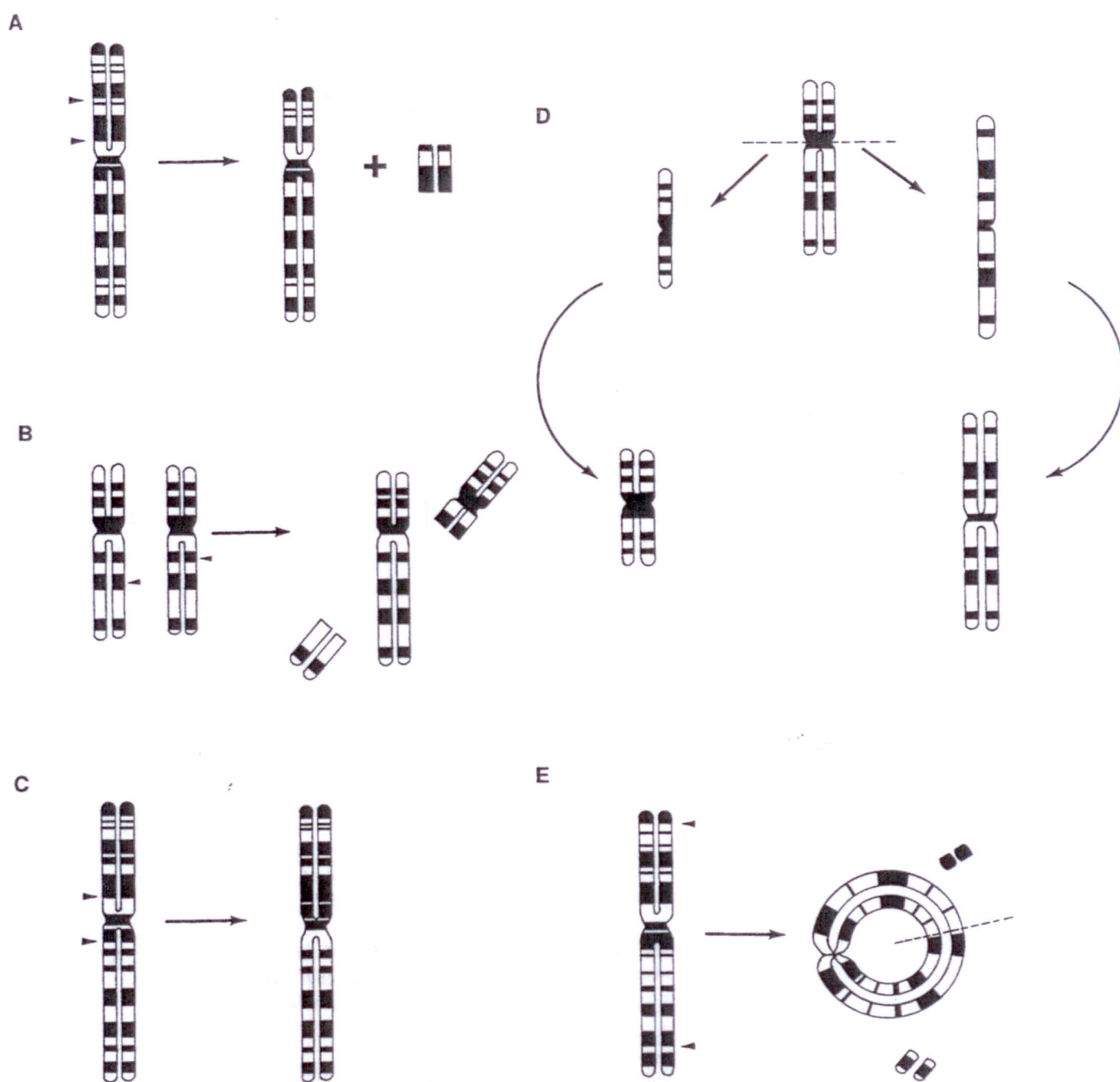


FIG. 17. Rearrangements involving a single chromosome or two homologs. **A:** Deletion; **B:** duplication; **C:** inversion; **D:** isochromosome formation; **E:** ring.

niques are too small for discovery by the usual cytogenetic methods (28,29). See Chapter 12 for more about molecular cytogenetics.

Duplication

A portion of one homolog may be present in duplicate. A duplicated segment may be inserted in the same order as the original segment (so that it is impossible to say

which piece was the original), or it may have become reversed. Tandem duplication (duplication in the same order, such as JK.LMN.LMN.OP) probably results from unequal crossing over in meiosis or from a rearrangement between two chromatids during mitosis. To form a reversed duplication the section must be inserted backward next to the original segment (JK.LMN.NML.OP). Various mechanisms have been postulated to account for this rearrangement, but the exact mechanism is not

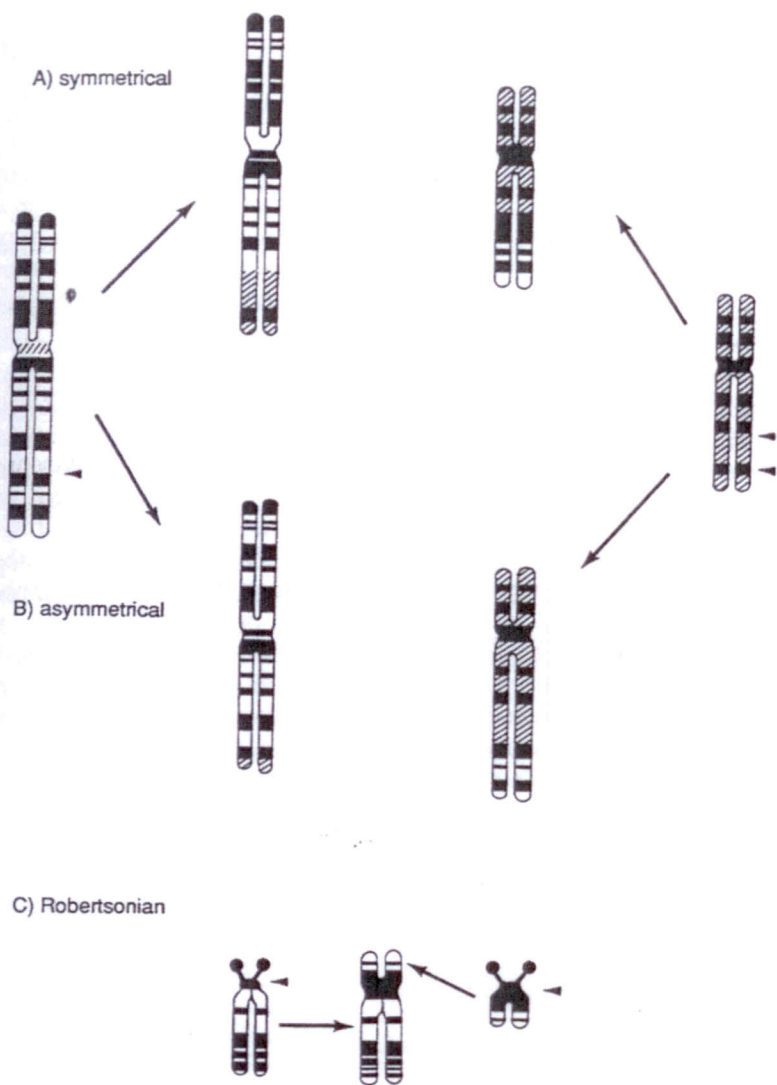


FIG. 18. Translocations. A: Symmetric; B: asymmetric; C: Robertsonian.

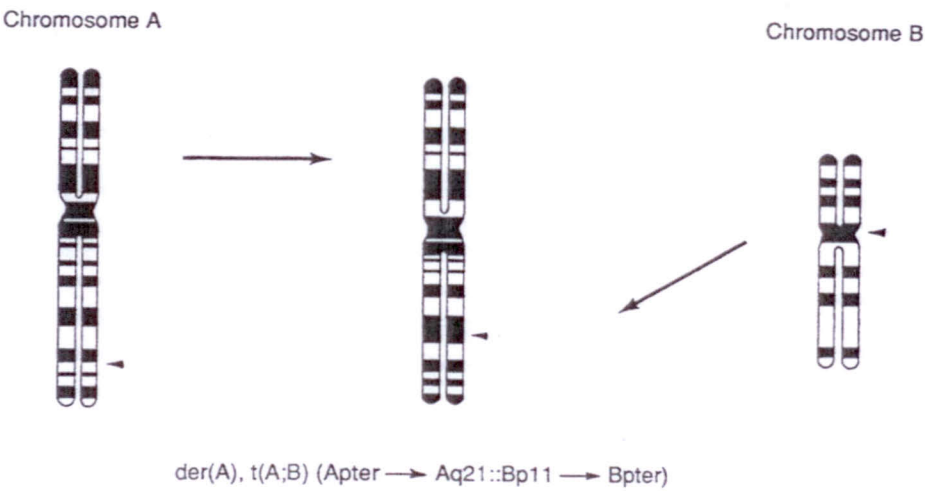


FIG. 19. Derivative chromosome from a balanced translocation.

known. Crossing over is known to occur during meiosis, and if it occurs between normal and rearranged chromosomes, it can lead to duplication of chromosome segments (86). Functionally, any duplication represents a trisomic state for whatever gene loci are present on the affected segment.

Inversion

Inversion is a two-break structural anomaly that affects a single chromosome. The segment between the two breaks rotates 180° and reattaches to the points of breakage. Inversions can be paracentric or pericentric. In paracentric inversions the centromere is not included in the inverted segment and the arm ratio of the chromosome is unchanged. Pericentric inversions are those in which the inverted portion does include the centromere and the arm ratio may be changed.

Chromosomal abnormalities can occur as a result of inversions; mechanical problems in pairing between a normal and an inverted chromosome during meiosis can lead to formation of a loop, and if crossing over occurs within the loop, a deleted or duplicated chromosome can result. However, because most inversions do not cause loss of genetic material, there may be no phenotypic effects. Inversions are only rarely implicated in chromosomal abnormalities in humans (87,88). Pericentric inversions are most common on chromosome 9 and, in this case, do not seem to cause any increased risk of miscarriage or of unbalanced offspring and so are considered normal variants (9).

Isochromosome

An isochromosome is a chromosome in which the arms on either side of the centromere are morphologically identical and bear the same gene loci. It was suggested in early publications that isochromosomes might be formed during cell division if the centromere separated the two arms rather than the two chromatids. This transverse fission of the centromere would lead to formation of one or two isochromosomes when, in subsequent mitoses, the joined arms each acted as a bi-armed chromosome. This type of centromeric fission is now considered unlikely (89). Most isochromosomes are thought to be formed by chromatid to chromatid exchange, or chromatid translocations within a chromosome, following breakage and loss of the distal sections of the chromatids. This would account for the finding that many isochromosomes are dicentric. Even isochromosomes that appear monocentric may have two centromeres so close that the separation is not apparent, or special stains may show that there is a suppressed centromere, and the chromosome is then termed a pseudodicentric. Further elucidation of the nature of centromeres by molecular methods might lead

to a better understanding of the variations possible in centromere formation.

The clinical effects manifested by isochromosome formation are a result of both a monosomic state for the loci that are lost and a trisomic state for the genes present on the isochromosome. Isochromosome formation of the q arm of chromosome X has been reported as a variant of Turner's syndrome. Isochromosome 17q is seen in some leukemia patients. Isochromosomes also occur with chromosomes 13, 21, and Y.

Ring Formation

Ring chromosomes are formed when simultaneous breakage occurs at two points on a single chromosome and the central portions are linked in a ring. The terminal fragments are usually lost, and the clinical effects reflect the monosomic state of these lost loci. Rings can have problems in mitosis, particularly if a twist develops in a ring through breakage and reunion. Cell lines may evolve with double-sized rings or with broken fragments of the ring. Rings have reportedly formed from all of the different chromosomes in the human genome. It has been suggested that the presence of a ring of any type can lead to ring syndrome (90) because of the random duplication and deletion of genetic material in the many different cell lines.

Rearrangements Involving More Than One Chromosome

Translocation

Translocation is an exchange of genetic material between two or more nonhomologous chromosomes. Such an exchange is not a normal mechanism but can occur if two or more chromosomes break at the same time. Broken chromosome ends are "sticky" and attract the cellular enzymatic repair service. Usually the broken ends stay close to each other and are reunited without any further trouble, but it is possible to mismatch the broken ends. Breakages tend to occur at fragile sites at or near the centromere or the chromosome ends and at euchromatic-heterochromatic junctions. A translocation is said to be reciprocal, or balanced, when no material is lost in the exchange. Translocations are defined morphologically in the following manner (see Fig. 18): symmetric translocations are those in which the translocated segments of the two chromosomes are of equal length, with no resultant change in arm ratio. In asymmetric translocations, the translocated segments have unequal length, and a change in morphology is evident. Robertsonian translocations occur when fusion of two acrocentrics in the region of the centromere results in one abnormal chromosome (91).

Translocations are the most common structural abnormalities in the human chromosome complement. Any

chromosome may be involved in a translocation rearrangement. Symmetric or asymmetric reciprocal translocations, which yield two or more functionally normal chromosomes without loss of genetic material, generally do not produce phenotypic effects, although there are exceptions (92). The carrier of a balanced translocation may pass on only part of the translocation, resulting in genetically unbalanced offspring (93,94). A translocation of either type can cause the carrier to be functionally sterile owing to the complex synaptic figures that occur during meiosis (95). Complex translocations involving rearrangements from more than two breaks can cause serious problems in cell division (96,97). Most of the partial trisomies reported in the section on chromosome variations are the result of unbalanced allocation of genetic material because of a parental translocation.

The problems of unbalanced gametes are important for genetic counseling and have been discussed in the literature (94,95,98). Small exchanges are more likely to produce viable dysmorphic infants, whereas large exchanges tend to lead to greater problems with spontaneous abortions (99–101). It may be that the presence of a complex translocation can lead to further meiotic problems, as described by Speevak et al. (102), in relation to a case of Down syndrome.

Sporadic translocations may be caused by the culture process itself. Translocations between chromosomes 7 and 14 have been reported as the most common of such translocations observed in PHA-stimulated lymphocyte cultures of normal individuals. Breakpoints were found at 7p13, 7q32-q35, 14q12, and 14q32 (103). Follow-up studies indicate that these sporadic translocations probably have no clinical significance, unless associated with other conditions such as ataxia-telangiectasia.

Derivative Chromosomes

The chromosomes that result from a translocation event are termed derivative chromosomes. The chromosomes are referred to by the number of the source centromere. For example, if there is a translocation between a chromosome 3 and a chromosome 6, there will be a derivative 3 chromosome and a derivative 6. The chromosomal constitution of the offspring is described by giving the breakpoints of the translocation and listing which derivative chromosomes are present (see Fig. 19). (See the nomenclature section of this chapter.)

Dicentric Chromosome

A dicentric chromosome possesses two centromeres and is the result of the joining of two broken pieces of chromosomes that each has a centromere. Dicentrics may be formed from two different chromosomes, such as a Robertsonian 14;21, by joining of homologous chro-

matids to form an isochromosome. Some dicentrics have so little separation of the centromeric material that the centromeres operate together as one very large centromere, functioning normally in cell division. Dicentrics may present problems during cell division, however, if the two centromeres are far apart on the derivative chromosome. If the centromeres are both active, they can be drawn to opposite poles of the spindle, resulting in formation of an anaphase bridge, a chromosome that makes a bridge between two daughter cells at anaphase. When this happens, the dicentric can be left outside both daughter nuclei as they form or can break apart and cause loss or gain of chromosomal material. In some dicentric chromosomes (pseudodicentric), one of the centromeres is suppressed; although the heterochromatic material can be demonstrated with special stains, it does not operate as a primary constriction and thus avoids complications at cell division. Such dicentric chromosomes are most likely to be observed in cancer cells and represent an acquired abnormality. The most common dicentric and pseudodicentric chromosomes are formed from the acrocentric D and G group chromosomes, though occasionally the other autosomes might be involved. Usually, in cells with a pseudodicentric chromosome, the same centromere appears to be suppressed in all cells, but this is not always the case. There is a case report in the literature of a patient having a *de novo* dicentric chromosome (5q;15q) that seemed to be variably pseudodicentric for either the 5 or the 15 centromere or to have both centromeres evenly constricted in some cells (104).

Insertion

Insertion is the addition of genetic material between two normally adjacent sections of a chromosome. The additional material could be a duplication of a section of the same chromosome but is more likely to come from a different chromosome. Insertions are difficult to identify unless there is a complementary deletion in the cell. Small insertions are now being identified by fluorescent chromosome painting techniques (see Chapter 13).

Marker Chromosome

A marker chromosome is abnormal, is morphologically different from the known chromosomes of the genome under study, but is contained in the cells being studied. A marker must have a centromere. It may be derived from breakage of a chromosome with loss of the acentric fragment and nondisjunction from its homolog during meiosis. Markers can also be isochromosomes or normal chromosomes with unusual heteromorphic variants such as inversions or extra heterochromatin (105,106).

The clinical significance of marker chromosomes varies widely. In some incidences, familial occurrence

and transmission of a small marker has been reported to have no phenotypic effects. However, a small metacentric marker identified as an isochromosome 18p produced severe clinical effects in another family (107). Several cases of marker chromosomes and their effects have been summarized by Nielsen et al. (108).

A small metacentric marker chromosome that had been variously identified as a partial 20, a partial 13, and an isodicentric 22 is associated with a set of symptoms classified as cat's eye syndrome (23,124). Using the more precise banding studies and better mapping techniques now available, researchers have reported the true identity of this chromosome to be a duplication of the centromeric region of chromosome 22 (pter to q11) (109).

Note that in writing a karyotypic description, if a marker chromosome has an identifiable centromere, it should be included as a derivative chromosome (der). The marker symbol (mar) should only be used if no further identification can be made (17).

Meiotic Nondisjunction

Because of the complicated nature of pachytene pairing in meiosis when a reciprocal translocation is involved, carriers of such translocations not only are likely to produce the obvious unbalanced gametes due to mispairing of the chromosomes involved (derivatives and the normal counterparts) but also are likely to have nondisjunction of these chromosomes, leading to even greater imbalance. This is particularly true for carriers of the 11;22 translocation. This occurrence is termed 3:1 meiotic nondisjunction because at the first anaphase of meiosis three of the affected chromosomes go to one cell and only one goes to the other cell (110,111).

Other Distinguishable Abnormalities Related to Chromosome Breakage

Breaks and Gaps

Fracture of chromosomes occurs at various stages of cell division, and most breaks are repaired without causing any abnormalities. Chromatid breaks and gaps occur in only one chromatid, but chromosome breaks and gaps occur in both chromatids at the same point (Fig. 20). Occasional chromosome breaks that are seen in the metaphase spread most likely represent random damage and usually are not clinically significant. A break is defined as the complete separation of a section of a chromosome such that a distinctly separate fragment is visible or is totally lost from the spread. A gap is formed when a section of a chromatid becomes attenuated so that there appears to be a discontinuity; the two adjacent regions are still connected to each other, but the thread of DNA is so fine that it seems invisible. Gaps are usually caused by some alteration in the fine structure of the DNA at the

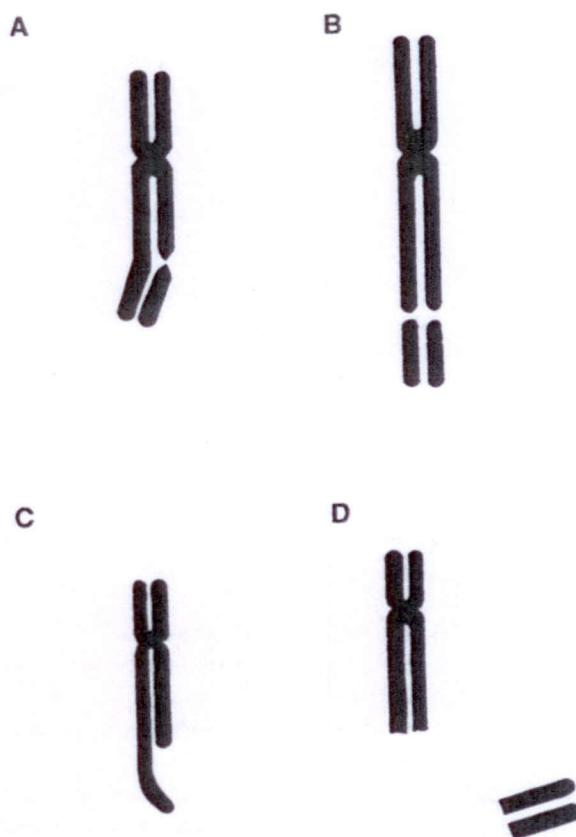


FIG. 20. Gaps and breaks. **A:** Gap in one chromatid—nothing missing. **B:** Gap in chromosome—nothing missing. **C:** Break in one chromatid—material missing. **D:** Break in chromosome—fragment displaced.

gap location, which affects the ability of the section to condense tightly and produce the metaphase form of the chromosome. Some gaps that are not usually observed can be induced by culturing cells in special culture media.

Breakage and gap formation are significant from a clinical point of view because minute pieces of chromosomal material or acentric fragments can be lost during the process. Also, broken chromosomes tend to unite readily with one another and can cause translocations, rings, and dicentric chromosomes. An increased rate of chromosomal breakage and gap formation might be indicative of a clinical condition. Such defects are known to occur in a group of syndromes known as the chromosomal breakage syndromes (112), which show increased chromatid exchange and quadriradial formation in Bloom's syndrome and increased rate of breaks and rearrangements or translocations in ataxia-telangiectasia, Fanconi's anemia, and xeroderma pigmentosum.

Fragile Sites Associated with Clinical Symptoms

A fragile site is described as a specific point on a chromosome with the following features. It is a nonstaining gap

of variable width that usually involves both chromatids; it is always found at the same band location on the same chromosome in the individual patient or kindred; and it is inherited in a Mendelian fashion. Fragile sites, including the fragile X, may be observed after culture of cells in specially formulated medium such as medium 199 (low in folic acid). They are usually expressed as an isochromatid gap, although some instances of spontaneous acentric fragments and triradial figures have been observed.

There are now more than a dozen well-documented heritable fragile sites in the human genome (113–116). Only the fragile X has been shown to be associated with a clinical disorder. Cytogenetic studies on any patient with mental retardation of unknown etiology should be regarded as incomplete if detection of the fragile site on the X chromosome at band Xq27.3 has not been attempted through appropriate culturing techniques (117–119). More information about the fragile X site has come from molecular studies, which are preferred for detection of this syndrome because of their greater precision (see also Chapter 12).

Chromosomal Breakage and Cancer

Chromosomal instability is a major factor in the development of some tumors (120). The types of rearrangements that can occur are described in Chapters 7 and 8.

CHROMOSOME VARIATION

Normal variation in chromosomes has been called polymorphic variation, although that term has been used for years with a different meaning by population geneticists. The proper term for cytogenetic variation (the term adopted by the Paris Conference) is heteromorphic variant. However, polymorphism is the preferred term among workers in cytogenetics and is the term more likely to be used. Although variations rarely have any clinical significance, they can be used for population studies, study of the origin of nondisjoined chromosomes (as in trisomy 21), paternity determination, detection of maternal contamination in amniotic fluid cultures, establishment of the origin of ovarian teratomas, determination of the zygosity of twins, and determination of the origin of certain deleted or translocated chromosomes (121). Variants can also be useful in bone marrow transplantation follow-up studies.

Variants seen without banding include secondary constrictions of chromosomes 1, 9, and 16. The length may vary on all three, and the position may vary on chromosome 9 if an inversion is present (36). The presence and size of the short arms of the acrocentric chromosomes can vary, and the long arm of the Y chromosome can be smaller than chromosome 21 or larger than chromosome 18. Special banding techniques such as C banding, fluorescent Q banding, or NOR silver staining can be used to

demonstrate other variations existing in the human chromosome complement (see Chapter 6).

CHROMOSOME DESCRIPTIONS AND KNOWN VARIATIONS

The clinical abnormalities in the following pages are listed by number of the affected chromosome. Almost all the abnormalities caused by trisomies or monosomies for parts of chromosomes are the result of unbalanced inheritance of translocation chromosomes. Clinical pictures and more detailed descriptions are given in de Grouchy and Turleau (122), and literature references are cited there and in Borgaonkar (81).

Refer to Fig. 3 for schematic diagrams of chromosomes at a band level of 550. Recall that in discussions of bands, both light and dark segments are bands; however, in the following descriptions of chromosomes, the dark bands of the Giemsa-stained preparations and the corresponding light bands of the fluorescent preparations are emphasized.

The Autosomes

Chromosome 1 (A Group)

Description and Landmark Bands

A. Conventional stain: Largest chromosome, metacentric.

B. G banding: Short arm (p): lighter at distal end

Long arm (q): dark heterochromatin might be visible just below the centromere; two distinguishable dark bands below, separated by a good-sized light band.

C. Q banding: Short arm (p): two proximal medium-bright bands; paler at distal end.

Long arm (q): negative band where heterochromatin block is located; central band intense, two distal bands less intense.

D. Giemsa R banding: Short arm (p): darker at distal end where G bands are light.

Long arm (q): proximal heterochromatin stains negatively; two light bands below correspond to the dark G bands.

E. C banding: Polymorphism: The large dark heterochromatin block (h region) under the centromere can vary greatly in size but is constant from cell to cell in an individual. This is regarded as a normal variant.

Clinical Abnormalities

Trisomy 1q32-qter causes severe malformations usually leading to early death; low birth weight; facial dysmorphism including wide forehead, hypertelorism, and

micrognathia; and severe mental retardation if the child survives. Its occurrence is rare.

Monosomy 1p36 has been reported recently (83,123), with eye problems and mental retardation. All cases were *de novo*.

Monosomy 1q4 shows microcephaly, generalized hypotonia, short stature, and severe mental retardation. Its occurrence is rare.

Ring 1 abnormalities cause low birth weight, considerably delayed development, and moderate mental retardation. The occurrence is rare, but survival to teens is possible.

Chromosome 2 (A Group)

Description and Landmark Bands

A. Conventional stain: Largest submetacentric chromosome.

B. G banding: Short arm (p): one dark band just above the centromere, and three more above that, almost equidistant from one another.

Long arm (q): two prominent double dark bands centrally, two distal, separated by one lighter, sometimes double, band.

C. Q banding: Short arm (p): medium fluorescence; central band in this arm slightly more intense.

Long arm (q): medium fluorescence throughout; two central bands intense.

D. Giemsa R banding: Short arm (p): broad, dark band at distal end.

Long arm (q): broad, dark band below centromere, which can separate into two in good preparations.

Clinical Abnormalities

Trisomy 2 mosaicism is occasionally seen as an artifact of culture of amniotic fluid samples.

Trisomy 2p2 and trisomy 2p3 cause severe craniofacial dysmorphism and severe retardation. Trisomy 2pter resembles Aarskog's syndrome, with short stature and skeletal anomalies. Their occurrence is rare.

Pericentric inversions have been observed in the region 2q1, which might lead to meiotic difficulties; they have been reported with both normal and abnormal phenotypes.

Chromosome 3 (A Group)

Description and Landmark Bands

A. Conventional stain: Metacentric; similar in length to the B group (chromosomes 4 and 5); larger than the C group.

B. G banding: Short arm (p): proximal area dark; dark band just before a terminal light band on the distal end.

Long arm (q): almost identical to the short arm pattern except that the distal dark band is twice as broad as its counterpart on the short arm.

C. Q banding: Same pattern as G, but with dark G bands appearing bright, with medium fluorescence intensity. Polymorphism: in some individuals, a bright centromeric band is observed with varying intensities: approximately 55% of the population has this bright variant, usually at the centromere or proximal long arm.

D. Giemsa R banding: Short arm (p): one dark band in the center, one smaller terminal band.

Long arm (q): one central dark band, one smaller terminal band.

E. C banding: Approximately 2% of the population show a variable C-positive band.

Clinical Abnormalities

Trisomy 3p2 causes a high forehead with frontal bossing, large mouth, and small chin; occasional congenital heart defect; and retardation (IQ about 50). Its occurrence is rare and is due to familial translocations.

Trisomy 3q2 resembles Cornelia de Lange's syndrome with hirsutism but is more likely to have ocular complications. Its severity and prognosis are directly correlated with the size of the q2 section involved. Cases reported are primarily due to familial translocations; in those cases due to parental inversion and recombination, the effects of the associated monosomy 3p seems to be overshadowed by the effects of the trisomy. Another ocular disease spectrum, blepharophimosis-ptosis-epicanthus inversus syndrome (BPES), has recently been mapped to 3q23 (124).

Monosomy 3p2 is seen with low birth weight, anomalous shape of skull, facial dysmorphism, some inner organ defects, and severe mental retardation. Its occurrence is very rare.

Chromosome 4 (B Group)

Description and Landmark Bands

A. Conventional stain: Very submetacentric; larger than the C group (chromosomes 6–12); indistinguishable from chromosome 5.

B. G banding: Short arm (p): dark band in the center, which can often be seen as double, distinguishing it from 5.

Long arm (q): distinguishing band just below the centromere, which either is not present or is smaller and lighter on chromosome 5.

C. Q banding: Same pattern as G, with dark G bands appearing bright, with medium-intensity fluorescence.

Polymorphism: A bright band can be seen near the centromere in 13–40% of the population, its rate of appearance depending on ethnic group. The band appears exactly at the centromere or on the proximal short arm.

D. Giemsa R banding: Short arm (p): distal dark telomeric band.

Long arm (q): slight dark band just below where the dark proximal G band would be seen; slight dark band toward distal end, followed by a light band and then a dark telomere.

E. C banding: Approximately 2% of the population show variation in the presence or size of the C band at the centromere.

Clinical Abnormalities

Trisomy 4p2 and 4p3 are associated with microcephaly, absence of nasal bridge, large round low-set ears, anomalies of the hand, cardiac malformation, horseshoe kidney, and mental retardation. Occurrence is rare and is primarily due to familial translocations, frequently with chromosome 18. Survival is usually limited, though long-term survival is possible.

Monosomy 4p appears with severe growth retardation; characteristic facial dysmorphism evocative of a Greek warrior helmet because of the continuation of the plane of the forehead into the nose; frequently, cleft lip and palate, microcephaly, downturned mouth, and small chin; and severe mental retardation. The occurrence is relatively rare, usually *de novo*, though it is sometimes caused by familial translocations. There are several patients living past their teen years, and life expectancy is unknown.

Ring 4 cases have symptoms similar to monosomy 4p if band p16 is missing. Otherwise, no clear syndrome is defined, except for severe limb defects. Occurrence is rare.

Chromosome 5 (B Group)

Description and Landmark Bands

A. Conventional stain: Very submetacentric; larger than C group; indistinguishable from chromosome 4.

B. G banding: Short arm (p): very dark band in center, which may have blurred edges.

Long arm (q): characterized by three bands that fuse in a dark area in the middle of this arm; area followed by a light band, a subterminal dark band (which may be split in two), and a light telomeric band.

C. Q banding: Similar to G pattern, with medium-intensity fluorescence.

D. Giemsa R banding: Short arm (p): very dark telomere.

Long arm (q): dark proximal area, then a broad light band followed by two dark bands at the distal end.

E. C banding: Polymorphism: 6% of the population have a large C-positive band.

Clinical Abnormalities

Trisomy 5p cases discovered are usually the result of a familial translocation. Severity of the symptoms seems to depend on the extent of the triplication. Occurrence is rare.

Trisomy 5q3 causes microcephaly, large eyes and small mouth, cardiac anomalies, and severe mental retardation. Its occurrence is rare.

Monosomy 5p, or cri-du-chat syndrome, is characterized by a shrill cry in the newborn period; microcephaly with moon-shaped face, hypertelorism, and micrognathia; cerebral anomalies; severe mental retardation; hypotonia; and diverse internal malformations of heart, kidney, and abdomen. The severity of the condition depends on how much of the short arm is missing. The occurrence is 1 per 50,000 live births; currently more than 500 cases have been reported. Many patients have attained adulthood. Most cases are *de novo*, though the possibility of a parental rearrangement should be investigated.

Ring 5 may have similarities to 5p- if enough of the short arm is missing. Its occurrence is extremely rare.

Chromosome 6 (C Group)

Description and Landmark Bands

A. Conventional stain: Submetacentric; largest of C-group chromosomes.

B. G banding: Short arm (p): broad light band in the middle.

Long arm (q): two dark bands in the center, relatively indistinct.

C. Q banding: Same pattern as G; medium intensity.

D. Giemsa R banding: Short arm (p): dark broad band in center.

Long arm (q): two dark bands in the upper half of the arm, and a dark telomere.

E. C banding: Polymorphisms: 1% of the population show a variable centromeric band. A G-negative, C-positive area on the proximal short arm might be present and in some cases might be very large (chromosome may appear almost metacentric).

Clinical Abnormalities

Trisomy 6p2 shows a striking eye deformity in all cases—blepharophimosis—with eyelids tightly closed or drooping; round face, short nose, low-set ears; severe growth retardation; and feeding difficulties. Occurrence is extremely rare.

Trisomy 6q2, is characterized by small stature; short, webbed neck; few anomalies in the newborn period, but skeletal and muscular problems developing with increasing age; and very severe mental retardation. Its occurrence is extremely rare.

Ring 6 causes low birth weight, microcephaly, micrognathia, short neck, and severe mental retardation. Occurrence is extremely rare.

Chromosome 7 (C Group)

Description and Landmark Bands

A. Conventional stain: Submetacentric, slightly smaller than chromosome 6.

B. G banding: Short arm (p): distinct, dark nearly terminal band, which often has a flattened appearance, light telomeric material distal to this band.

Long arm (q): Two very distinct dark bands, followed by a third, less intense dark band and a light telomere.

C. Q banding: Same pattern as G; medium-intensity fluorescence on short arm; slightly more intense bright bands on the long arm.

D. Giemsa R banding: Short arm (p): dark band at telomere followed by light band; the rest of the arm dark overall.

Long arm (q): dark band under the centromere, followed by two separate light bands; the rest dark overall.

E. C banding: Polymorphism: Between 4% and 10% of the population display a larger C-banded centromere, possibly because the centromere is bipartite and because it is normally slightly larger than those on other C-group chromosomes (except for chromosomes 9 and 11).

Clinical Abnormalities

Trisomy and monosomy have been reported for small regions of both p and q arms, but they are extremely rare. Abnormalities are severe in these cases, leading to early death. These cases usually occur as the result of unequal crossing over after translocation events.

Chromosome 8 (C Group)

Description and Landmark Bands

A. Conventional stain: Very submetacentric, similar to chromosomes 10 and 12.

B. G banding: Short arm (p): two narrow dark bands with an almost equal-sized light band between them.

Long arm (q): two dark bands: the first in the center of the arm, and the second, usually darker, band more distal.

C. Q banding: Same pattern as G; medium-intensity fluorescence.

D. Giemsa R banding: Short arm (p): dark telomere, relatively dark overall.

Long arm (q): dark telomere and dark band about 1/3 of the distance from telomere. Light band between is equivalent to distal dark G band.

E. C banding: Polymorphism: 1% of the population has an enlarged centromeric band.

Clinical Abnormalities

Trisomy 8 (mosaic), Warkany's syndrome, causes a long face, with thick everted lower lip; large ears; deformed or absent patella; and a deep cleft on the sole of the foot between the ball and other toe joints; but few other physical abnormalities (67). Some mental retardation is possible, but some patients are sufficiently normal to escape detection until adulthood. The occurrence is possibly 1 per 100,000 live births; the syndrome affects more males than females.

Trisomy 8p is characterized by a high forehead, wide face with chubby cheeks, fleshy everted lower lip, and large ears. Occurrence is rare. Some cases occur *de novo*, as direct or mirror duplications; others are due to familial rearrangements.

Trisomy 8q2 correlates with a prominent forehead, small receding chin, cardiac anomalies, skeletal problems, and mental retardation. Its occurrence is rare and is usually due to parental rearrangements.

Monosomy 8p2 appears with a small skull, short nose, small mouth, cardiac defects, and language difficulties. Occurrence is extremely rare; most cases are *de novo*.

Chromosome 9 (C Group)

Description and Landmark Bands

A. Conventional stain: Varies from slightly submetacentric to very submetacentric, depending on the amount of heterochromatin located in the region just proximal to the centromere on the long arm. This area may also stain lightly and may appear stretched.

B. G banding: Short arm (p): quite dark overall, with a central darker band. Good banding on longer chromosomes will show two distinct bands.

Long arm (q): a variable, proximal, thin light area may be present—it can be as long as the short arms or almost nonexistent without having any noticeable phenotypic effect; this region is called the secondary constriction. A heavy dark band is distal to the secondary constriction, followed by two smaller dark bands.

C. Q banding: Same pattern as G; medium-intensity fluorescence. The distal bright band on the long arm rarely shows the subbands.

D. Giemsa R banding: Short arm (p): dark band above centromere with light stain on the remainder.

Long arm (q): the secondary constriction stains lightly. This is followed by a fine, dark band; a broad, light band; then two dark bands, the second of which is telomeric.

E. C banding: Excellent for polymorphism studies owing to the variability of the size and position of the heterochromatic region. Approximately 8% of the population have increased heterochromatic material; 5% have a very small or absent area; and 3% have a pericentric inversion involving all or part of the region.

Clinical Abnormalities

Trisomy 9 has been reported, both homogeneous and mosaic, but is very rare. Trisomy 9p is more common, usually arising *de novo* but occasionally appearing from a parental rearrangement. Tetrasomy 9p, resulting from the presence of an isochromosome 9p, has also been reported. Trisomy 9q3 has been reported, arising from a *de novo* tandem duplication, and a very few cases have been noted from familial translocations.

Ring 9 has been reported, with variable phenotype depending on the quantity of genetic material missing.

Chromosome 10 (C Group)

Description and Landmark Bands

A. Conventional stain: Submetacentric, similar to chromosome 8.

B. G banding: Short arm (p): dark overall with a small dark band at the center.

Long arm (q): prominent distinguishing dark band proximally, followed by two dark equidistant bands, the second of which may appear terminal but is actually followed by a light band, often unclear.

C. Q banding: Same pattern as G. The proximal band on the long arm is more intense than the others.

D. Giemsa R banding: Short arm (p): darkly staining overall.

Long arm (q): four dark bands appear, one next to the centromere, the others corresponding to the light areas seen with trypsin G bands.

F. C banding: Dark centromere, but no other heterochromatic regions.

Clinical Abnormalities

Trisomy 10p appears with growth retardation, muscular hypoplasia, and hypotonia; cleft lip and palate; cardiac and renal malformations; bone anomalies; and severe mental retardation. Occurrence is very rare and is usually due to parental translocations.

Monosomy 10p shows severe malformations of organs and skeleton. Its occurrence is extremely rare and all cases are *de novo*.

Ring 10 causes cardiac and renal anomalies. Occurrence is extremely rare.

A pericentric inversion transferring the large dark band at q21 to the short arm has been reported.

Chromosome 11 (C Group)

Description and Landmark Bands

A. Conventional stain: Submetacentric, but closer to metacentric than the other C group chromosomes. The area under the centromere may appear as a secondary constriction.

B. G banding: Short arm (p): strong, sometimes double band just below the center, which fades out distally into a thin telomere.

Long arm (q): A proximal dark band distinguishes this chromosome from chromosome 9. This is followed by a broad, light band; a central, broad, double, dark band; and a light telomere.

C. Q banding: Same pattern as G; medium-intensity fluorescence.

D. Giemsa R banding: Short arm (p): tiny proximal dark band; center is light; telomere is dark.

Long arm (q): dark proximal band, followed by a broad light band and a dark telomere.

E. C banding: Polymorphism: 13% of the population show an enlarged C band, similar to that sometimes seen on chromosome 7.

Clinical Abnormalities

Trisomy 11p appears with a high, convex forehead with upswept hair; hypertelorism with a wide nasal bridge, round chubby cheeks, and cleft lip and palate; hypotonia; and severe mental retardation. Its occurrence is extremely rare, resulting from complex translocations.

Trisomy 11q2 causes a long philtrum, retracted lower lip, microretrognathia, growth retardation, and early death. Occurrence is rare. Cases are frequently due to a parental 11;22 translocation and 3:1 meiotic segregation; other cases are due to parental translocation to a chromosome other than 22.

Monosomy 11q13, or Wilms' triad, shows aniridia; ambiguous genitalia; mental and growth retardation; and nephroblastoma or gonadoblastoma. Occurrence is rare; usually it is a *de novo* deletion.

Monosomy 11q2 causes striking trigonocephaly; large mouth, receding chin; large, low-set ears; cardiac and renal anomalies; and severe mental retardation. Occurrence is rare and is usually *de novo*.

Chromosome 12 (C Group)*Description and Landmark Bands*

A. Conventional stain: Very submetacentric; usually the shortest short arms in the C group.

B. G banding: Short arm (p): dark central band.

Long arm (q): Proximal dark band followed by a light band, narrower than that on chromosome 11, then several dark bands very close together; a light telomeric region, broader than on chromosome 11.

C. Q banding: Same pattern as G, with medium-intensity fluorescence.

E. Giemsa R banding: Short arm (p): proximal dark band, light middle, dark telomere.

F. C banding: Polymorphism: 1% of population displays a larger C-positive region.

Clinical Abnormalities

Trisomy 12p cases are plump hypotonic infants with turreted skulls and severe mental retardation. Occurrence of the aberration is rare and is due to parental translocations.

Trisomy 12q2 causes a large head on a small body with short limbs and severe mental retardation. Its occurrence is extremely rare.

Monosomy 12p appears as microcephaly; small size and low birth weight, micrognathia and small pointed nose, and severe mental retardation. Occurrence is extremely rare.

Chromosome 13 (D Group)*Description and Landmark Bands*

A. Conventional stain: Large acrocentric, indistinguishable from chromosomes 14 and 15.

B. G banding: Short arm (p): might or might not show short arms and/or satellites of variable size.

Long arm (q): proximal half of arm light but divided in two by a narrow dark band. Distal half has two prominent dark bands separated by a narrow light band, followed by a light telomeric region with a small dark band in it.

C. Q banding: Same pattern as G; medium-intensity fluorescence. Polymorphism: approximately 50% of the population show bright intensity at the centromere. Satellites show varying intensity and size.

D. Giemsa R banding: Short arm (p): might or might not show short arm and satellites.

Long arm (q): dark proximal region, very light area followed by telomeric dark band.

E. C banding: Polymorphism: approximately 4% of the population show variable centromeric staining.

G. AgNOR stain might show silver deposits on short arm.

Clinical Abnormalities

Trisomy 13 causes severe disturbance of development, affecting the brain, heart, and kidneys as well as facial development (with the frequent occurrence of cleft lip and palate and severe hypotelorism) and development of extra fingers and toes. The disorders are usually sufficiently severe to lead to early death. The occurrence is about 1 in 8000–10,000 live births. A free chromosome 13 is involved in 80% of cases, and the rest are due to translocations (usually of the Robertsonian type to a chromosome 14 or 21, but occasionally to another 13 or to a 15). Occurrence of the translocation type can be caused by inheritance of a parental rearrangement or can be *de novo*.

In monosomy 13q14, the deletion occurs *de novo* and is probably due to unequal meiotic crossing over. Deletions can extend from q11 to q22 and have variable symptoms, but the common features of retinoblastoma and absence of band q14 characterize this group of cases. Occurrence is rare. Waardenburg's syndrome has also been linked to a deletion of 13q (125), and a linkage to Hirschsprung's syndrome is postulated.

Chromosome 14 (D Group)*Description and Landmark Bands*

A. Conventional stain: Large acrocentric, indistinguishable from chromosomes 13 and 15.

B. G banding: Short arm (p): might or might not show short arm and/or satellites.

Long arm (q): two distinguishing dark bands, one proximal and the other distal but subterminal.

C. Q banding: Same pattern as G; medium-intensity fluorescence.

D. Giemsa R banding: Short arm (p): might or might not show short arm and/or satellites.

Long arm (q): medium-dark broad proximal band followed by narrow light band, larger dark region, a second narrow light band and a dark telomere.

E. C banding: Polymorphism: approximately 5% of the population show variation of size of centromere and satellites.

F. AgNOR stain might show silver deposits on short arm.

Clinical Abnormalities

Trisomy 14 is characterized by an asymmetric face, short bulbous nose, small receding chin, low-set ears, and

ptosis of one or both eyes; heart disease; and severe mental retardation. Occurrence is extremely rare and is reported as low-level mosaicism.

Ring 14 causes dolichocephaly with a high forehead and receding chin; large, low-set ears and a short neck; heart defects; and mental retardation. Occurrence is extremely rare.

Chromosome 15 (D Group)

Description and Landmark Bands

A. Conventional stain: Large acrocentric, indistinguishable from chromosomes 13 and 14.

B. G banding: Short arm (p): might or might not show short arm and/or satellites.

Long arm (q): may show two bands like chromosome 14. The first one is almost central, and the second, if present, is terminal.

C. Q banding: Same pattern as G; medium intensity. Polymorphism: approximately 1% of the population have a large short arm; 10% have satellites of varying size and intensity of fluorescence.

D. Giemsa R banding: Short arm (p): might or might not have short arm or satellites.

Long arm (q): proximal third stains lightly; distal portion stains darkly.

E. C banding: Polymorphism: 5% of the population has enlarged paracentromeric region; 3% has variation in size of satellites.

F. AgNOR stain might show silver deposits on short arm.

G. The short arm has a 5-methylcytosine-rich band, which can be demonstrated by staining with fluorescein-tagged anti-5MeC or distamycin/DAPI.

Clinical Abnormalities

Trisomy 15q11 is characterized by an oval face; deep-set eyes; high cheekbones; short, thick fingers; severe mental retardation; and seizures. Its occurrence is rare. Tetrasomy for the same region is reported also, with formation of a bisatellited marker having two copies of the region.

Monosomy 15q11, Prader-Willi syndrome, shows neonatal hypotonia and poor feeding; short stature; obesity in later life. Occurrence is rare and is thought to be due to unequal meiotic crossing over, leading to the deletion of band 15q11. This deletion has not been demonstrated in all cases of Prader-Willi syndrome; sometimes it can only be demonstrated using fluorescent probes, and sometimes the cause is not deletion but uniparental odisomy. Angelman syndrome has also been mapped to this area. Both syndromes have associated mental retar-

dation. Note that isodisomy cannot be detected by conventional cytogenetic methods but requires use of DNA techniques (126).

Ring 15 causes severe growth retardation, both before and after birth, microcephaly, and variable psychomotor retardation. Occurrence is rare; it has been reviewed by Yunis (127).

Chromosome 16 (E Group)

Description and Landmark Bands

A. Conventional stain: Varies from almost metacentric to submetacentric, owing to a block of heterochromatin on the long arm adjacent to the centromere; usually similar in size to chromosomes 17 and 18.

B. G banding: Short arm (p): one central dark band with flanking light bands.

Long arm (q): Constitutive heterochromatin stains very darkly; two more dark bands below heterochromatin block; distal dark band appears terminal.

C. Q banding: Same pattern as G. Heterochromatin stains negatively.

D. Giemsa R banding: Short arm (p): entirely dark.

Long arm (q): proximal heterochromatin block is light. The rest of the long arm is dark, with a hint of a light band in the center.

E. C banding: Polymorphism: heterochromatin below centromere varies from small band to large block. Pericentric inversions are rare, but not unknown.

F. Other polymorphisms: There is a report of a family with an additional G-negative band on 16p that seems to have no phenotypic effects (128).

Clinical Abnormalities

Trisomy 16 occurs in some early spontaneous abortions but has not been reported in liveborn infants.

Trisomy 16p causes small round head, micrognathia, thumb anomalies, and severe growth and mental retardation. Occurrence is rare, and most cases are due to parental translocations.

Trisomy 16q is characterized by malformation of the head and extremities and by heart defects. Occurrence is rare, with all cases due to parental translocations and causing early death.

Chromosome 17 (E Group)

Description and Landmark Bands

A. Conventional stain: Same size as chromosome 18, but less submetacentric.

- B. G banding: Dark-staining centromere.
- Short arm (p): prominent central dark band.
- Long arm (q): light with two subterminal dark bands; hard-to-see light telomeric band.
- C. Q banding: Same pattern as G; medium-intensity fluorescence; pale short arms.
- D. Giemsa R banding: Short arm (p): uniformly dark.
- Long arm (q): dark bands proximally and distally, with lighter band between.
- E. C banding: Polymorphism: increased heterochromatic stain at centromere in 2% of population.

Clinical Abnormalities

Trisomy 17q22 causes facial dysmorphism with squinty eyes, large mouth, short neck; growth retardation; and cerebral, cardiac, and urinary tract malformations. Occurrence is very rare and is due to parental translocation.

Monosomy 17p13.3 is reportedly associated with Miller-Dieker syndrome (129). Clinical symptoms include microcephaly, small facies, retarded motor development, failure to thrive, and, usually, early death (14). Autopsies have shown severe abnormalities of brain (lissencephaly), kidney, heart, and digestive tract. Occurrence is extremely rare and is usually *de novo*, although one report shows that the deletion can be associated with a parental translocation (130).

Monosomy 17p11.2-p12: deletion of this small section of 17p was reported in a child with manifestations of Smith-Magenis syndrome, which include brachycephaly, midface hypoplasia, and delayed motor and social development. It was found that the mother, who showed minimal signs of the syndrome, was mosaic for the deleted chromosome 17 (85).

Chromosome 18 (E Group)

Description and Landmark Bands

- A. Conventional stain: Similar in size to chromosome 17, but more submetacentric; often the same shape as chromosome Y.
- B. G banding: Short arm (p): light, with a medium-intensity band.
- Long arm (q): Two characteristic dark bands—one proximal, one distal. There is a narrow light telomere that is hard to distinguish.
- C. Q banding: Same pattern as G; medium intensity.
- D. Giemsa R banding: Short arm (p): uniformly dark.
- Long arm (q): Proximal light band; dark central band; dark distal tip.
- E. C banding: Polymorphism: variable enlarged centromere in approximately 4% of the population.

Clinical Abnormalities

Trisomy 18, Edwards' syndrome, is characterized by severe growth and mental retardation; microcephaly, with micrognathia; overlapping fingers, narrow pelvis, rocker-bottom feet. The occurrence, about 1 per 1500 live births, is not as frequent as that of trisomy 21. About 80% have homogeneous trisomy, and the others are mosaic or are the result of parental translocations.

Monosomy 18p causes small size, round face, wide downturned mouth, and large ears; and cerebral, ocular, and cardiac anomalies. The occurrence is infrequent (more than 70 cases have been reported). About one third of cases are *de novo*, and the others result from parental translocations.

Monosomy 18q22 results in severe hypotonia with frog-like flexed position of legs, growth retardation, microcephaly, and moderate to severe mental retardation. Its occurrence is rare; it frequently occurs *de novo*, but a few cases come from parental translocations.

Most features of ring 18 are similar to those of monosomy 18q22.

Chromosome 19 (F Group)

Description and Landmark Bands

- A. Conventional stain: Smallest metacentric, indistinguishable from chromosome 20.
- B. G banding: Short arm (p): lightly stained chromatin with narrow dark band in center.
- Long arm (q): Same as short arm, but with a proximal dark band, which can appear as part of the centromere.
- C. Q banding: Same pattern as G; chromosome pale with medium intensity at the centromere.
- D. Giemsa R banding: Chromosome uniformly dark except at the centromere and at the heterochromatic band just below it.
- E. C banding: Polymorphism: varying size of the centromere and proximal heterochromatin.

Clinical Abnormalities

Trisomy 19q is characterized by low birth weight, microcephaly with flat face and hypertelorism, downturned mouth, small hands and feet, and growth and mental retardation. Its occurrence is very rare; cases result from parental translocations.

Chromosome 20 (F Group)

Description and Landmark Bands

- A. Conventional stain: Smallest metacentric, indistinguishable from chromosome 19.

B. G banding: Short arm (p): dark terminal band.

Long arm (q): Two narrow dark bands.

C. Q banding: Same pattern as G; medium intensity.

D. Giemsa R banding: Short arm (p): lighter stain on distal end than that on chromosome 19.

Long arm (q): Darker than short arm; centromere light and not as obvious as that on 19.

E. C banding: Polymorphism: variable C-band size, enlarged in 3% of the population.

Clinical Abnormalities

Trisomy 20 occurs in the mosaic state in amniotic fluid cultures but is very rarely found in the resultant child. Its occurrence is rare.

Trisomy 20p is characterized by a round face, high cheekbones, possible internal anomalies, and mental retardation. Occurrence is rare; all reported cases are due to familial translocations.

Chromosome 21 (G Group)

Description and Landmark Bands

A. Conventional stain: Acrocentric, indistinguishable from chromosome 22.

B. G banding: Short arm (p): possible short arms and/or satellites.

Long arm (q): Proximal region which dominates, fading out to almost invisible light telomere.

C. Q banding: Same pattern as G; intense long arm proximal band; varying size and intensity of satellites, if present.

D. Giemsa R banding: Long arm (q): proximal area light; distal area very dark.

E. C banding: Polymorphism: variable stain possible at the centromere or the satellites.

F. AgNOR: Silver deposits at location of NORs.

Clinical Abnormalities

Trisomy 21, or Down syndrome, is the most frequent autosomal anomaly, with characteristic facies; hypotonia; broad square hands with shortened fifth finger; broad, small feet with short toes and a large gap between the first two toes; cardiac defects; low birth weight; and mental retardation. It occurs in 1 in 700 live births, with an increased occurrence in older mothers. Of all cases, 92.5% are homogeneous trisomy 21; 4.8% are cases due to translocations, most arising *de novo* and almost all involving translocations with other acrocentrics, and the remaining are mosaic trisomies.

Ring 21 has several features in countertype to trisomy 21, including hypertonia, protuberant nasal bridge, and

severe internal anomalies. It is extremely rare; all reported cases were *de novo*.

Chromosome 22 (G Group)

Description and Landmark Bands

A. Conventional stain: Acrocentric; indistinguishable from chromosome 21.

B. G banding: Possible short arms and satellites.

Long arm (q): Dark centromeric region; light arm with dark central band.

C. Q banding: Same pattern as G; pale fluorescence with a bright centromere.

D. Giemsa R banding: Possible short arms and satellites.

Long arm (q): Dark with a hint of a lighter band in center.

E. C banding: Polymorphism: in short arm and satellites.

F. AgNOR: Silver deposits at location of NORs.

Clinical Abnormalities

Trisomy 22 is probably lethal; it is frequently observed in spontaneous abortion material. There are rare reports of mosaic trisomy 22 with very short survival.

Tetrasomy 22p, or cat's eye syndrome, is characterized by anal atresia, ocular coloboma, microphthalmia, congenital heart disease, and skeletal and urinary tract anomalies. Its occurrence is rare. Patients appear to be mosaic for an isochromosome 22p, bisatellited and frequently dicentric. However, this marker chromosome is not demonstrable in all cat's eye patients.

Monosomy 22q11, DiGeorge syndrome, has a very variable presentation, which may include cardiac defects, cleft palate, coloboma, deafness, and learning problems (131).

Ring 22 cases show severe mental retardation, hypotonia, excitability, and poor motor coordination. Occurrence is rare; some cases are mosaic.

Offspring of parents with t(11;22) are at risk for partial trisomy 22q.

The Sex Chromosomes

Chromosome X (Sex Chromosome)

Description and Landmark Bands

A. Conventional stain: Submetacentric, size and shape similar to that of chromosome 7. The late-replicating X is often bent or curled over, even if all the other chromosomes are straight, and may appear more condensed and seem not to pair very well with the active X.

B. G banding: Short arm (p): strong dark band in center. Long arm (q): the proximal dark band is about the same distance from the centromere as is the short arm dark band. A second dark band is often distinguishable, distal to the first band and less dark. There may also be a narrow terminal dark band.

C. Q banding: Same pattern as G; medium-intensity fluorescence.

D. Giemsa R banding: Short arm (p): dark with central light band

Long arm (q): dark except for proximal light band, corresponding to dark band in G pattern.

E. C banding: Polymorphism: Approximately 1% of the population has an enlarged C band.

F. Staining for late-replicating X: BrdU can be used for a pulse, either late or early in replication, and special stains will then distinguish the late- and early-replicating X chromosomes (see Chapter 6).

Clinical Abnormalities

Trisomy X may cause slight mental retardation and a slight tendency to schizophrenia. In most patients, phenotype, puberty, and fertility are normal. Its occurrence is about 1 per 1200 live female births, more frequent than monosomy X because it does not have the same tendency to cause spontaneous abortion. These patients are not usually detected at birth but are frequently found through familial studies of other chromosomal problems. Triple-X females may have a slightly greater tendency to meiotic nondisjunction, leading to birth of children with other trisomies.

Multiple-X karyotypes 48,XXXX and 49,XXXXX have been reported but are very rare. Mental retardation is usually increasingly severe with increased sex chromosome complement.

Monosomy X (Turner's syndrome) has various characteristics. In the infant, small size, lymphedema of the hands and feet, excess skin at nape of neck, and coarctation of the aorta are common, and in the adolescent girl, small stature, lack of secondary sexual characteristics, webbed neck, low hairline on neck, and anomalies of joints of arms and legs are characteristic. It occurs with a liveborn frequency of about 1 per 2000 female births but is more frequent in spontaneously aborted fetuses. Homogeneous 45,X is the most frequent chromosomal form (55%); mosaicism with a normal cell line or with trisomy X occurs in 10% of cases. The presence of an isochromosome of the long arm, i(Xq), in all cells or mosaically with normal cells or 45,X cells, accounts for a further 20% of cases. Other chromosomal variations that make up the final 15% of cases include deletions of the short arm of an X without isochromosome formation, either homogeneously or in mosaic form, and ring X.

Monosomy Xp (46,X,del(X)(p)) is clinically very similar to Turner's syndrome.

Monosomy Xq (46,X,del(X)(q)) is quite different in physical presentation. There is no growth retardation, and there are well-developed secondary sexual characteristics. However, the ovaries are not developed (streaks), and the patient is sterile. The occurrence is very rare.

The physical development of the male phenotype in 46,XX males generally appears normal, and these patients may often be detected only through infertility studies. Three reasons have been postulated for this phenotype: undetected mosaicism of male and female cells; translocation of the active portion of the Y chromosome to some other site in the genome; and gene mutation such that Y chromosomal control is no longer necessary to activate male-determining genes. It is now possible to use DNA probes of the Y chromosome genes to search for translocated Y chromosomal material, and such material has been found in some, but not all, cases. Further work on these patients is still being done. Occurrence of XX males is relatively rare (132).

Fragile X and mental retardation cases show facial dysmorphism with high forehead, long face, and thick lips; large ears; large testes and penis in males, particularly after puberty; and mental retardation and language difficulties. Occurrence is estimated to be about 2 per 1000 male births. The syndrome has also been noted occasionally in females, presumably because of the inactivation of the nonfragile X chromosome during embryonic development. The fragile site is located on the long arm at q27.3 and can sometimes be demonstrated by growth of cells in special culture medium that is deficient in folic acid. Although the tendency to breakage is known to be inherited and must be passed to the male through the female, this tendency can be difficult to demonstrate in all female obligate carriers. Even in studies of males, many cells might have to be examined, and a diagnosis is not always confirmed. The syndrome can be delineated better through molecular testing with DNA probes and PCR methods (133).

Chromosome Y (Sex Chromosome)

Description and Landmark Bands

A. Conventional stain: Generally very submetacentric, with very small short arms. The long arm is variable owing to the presence of constitutive heterochromatin, which may be deleted or duplicated with no phenotypic effect; Y can be smaller than a 21 or as large as a 13.

B. G banding: Short arm (p): lightly stained.

Long arm (q): Usually a small, less densely stained region just below centromere, and very dark to the terminal end; size of dark region dependent on the quantity of heterochromatin.

- C. Q banding: Short arm (p): pale fluorescence.
Long arm (q): Proximal region pale; heterochromatin in distal portion stains brilliantly. Polymorphism: the size of the brilliantly stained region varies according to the quantity of heterochromatin present.
- D. Giemsa R banding: Short arm (p): dark.
Long arm (q): very light overall.
- E. C banding: Polymorphism: The dark-staining heterochromatic region at the distal end of the long arm (q12) can vary in size from extremely small to more than 50% of the chromosome, without any phenotypic effects being observed.
- F. Lateral asymmetry stain: may show two or three regions in the heterochromatin (q12).

Clinical Abnormalities

47,XXY, Klinefelter's syndrome, is characterized by gynecomastia, testicular atrophy, sterility, and mild to moderate mental retardation. Patients are usually tall and may have psychological problems. Occurrence is approximately 2 per 1000 male births, in both homogeneous and mosaic forms. Patients are not usually detected before puberty (79).

Cases of 47,XYY have a variable clinical picture and may have no phenotypic symptoms. The occurrence of 47,XYY is estimated to be similar to that of Klinefelter's and triple-X syndromes, at 1-2 per 1000 live births. Because of the lack of physical features, many cases are likely to go undetected.

Individuals with 48,XXXY, 48,XXYY, and 49,XXXXY aberrances have been reported, but such cases are extremely rare. In general, the clinical picture is similar to that of 47,XXY, with increasingly severe mental, physical, and psychological problems (134).

Testicular feminization, caused by deletion of testis-determining factor on Yp or by mutation in autosomal genes that normally respond to the factor, characterizes 46,XY females: sterility and gonadal dysgenesis are effects. The occurrence is rare.

Abnormal and variant forms of the Y chromosome have been reported, including ring, deleted long arm, pericentric inversion, isochromosome of the long arm, translocations to other chromosomes (135), and satellites on either the long or the short arm.

True Hermaphroditism and Pseudohermaphroditism

These intersexual states are extremely rare (51,136,137). Clinically, hermaphroditism is characterized by ambiguity of the internal and the external genitalia. Histologic study of the gonadal tissue will reveal both male and female structures in the true hermaphrodite but tissue of only one sexual type in the pseudohermaphrodite. Cytogenetic studies are usually performed on blood and gonadal tissue to

determine karyotypic sex. The karyotype can be normal of either sex or, more rarely, a mosaic of male and female (sometimes involving a 45,X or a 47,XXY cell line). These are cases where the use of fluorescent probes and whole-chromosome paints can be extremely helpful in determining the presence of sex chromosomal material, either as whole chromosomes or in translocations of small pieces.

Ambiguous genitalia can sometimes arise in the newborn because of defects of development, and cytogenetic studies are usually ordered before performing any necessary surgery. These cases have to be considered individually because of the complexity of the surgical procedures that may have to be undertaken. In performing the cytogenetic studies, great care must be taken to ascertain or eliminate significant mosaicism. A comprehensive and compassionate review of these patients is given in Money and Ehrhardt (138).

It is interesting to note that adults requesting sex change operations generally do so for psychological reasons and are not discordant in their original development.

TABLE 2. Common nomenclature symbols and abbreviation

Symbol or abbreviation	Description
arrow	
→	from — to
colon, single	
:	break
colon, double	
::	break and rejoin
minus	
-	loss of material
parentheses	
()	enclose structurally altered chromosome
plus	
+	gain of material
question mark	
?	origin unknown
semicolon	
;	separates region of structural alteration
slant line	
/	separates cell lines in describing mosaicism
add	additional material of unknown origin
cen	centromere
del	deletion
der	derivative chromosome, result of a translocation
fra	fragile site
inv	inversion
mar	marker chromosome
mat	maternal origin
p	short arm of chromosome
pat	paternal origin
q	long arm of chromosome
rob	Robertsonian translocation
s	satellite
t	translocation
ter	terminal end of chromosome

NOMENCLATURE

It was realized very early in the history of cytogenetics that an international system of nomenclature would be necessary for normal and abnormal cases to be categorized and for sensible communication between investigators to be possible. The first conferences were held in Denver (1960), London (1963), and Chicago (1966) before the discovery of banding techniques. The original system of nomenclature was based on nonbanded chromosomes, describing and organizing them by size, centromere location, and arm ratio. The major conference that produced the system in use today was held in Paris in 1971, after the discovery of banding techniques. At this conference the original system of nomenclature was refined and expanded by the addition of a standardized method for identifying banded chromosomes that describes their appearance in both fluorescent and non-fluorescent stains (14–17). Symbols and abbreviations were given standard definitions and rules for usage (Table 2). Symbols were used to indicate variation from the normal quantity of genetic material and to show relationships between sections of chromosomes such as breakage and reunion. Abbreviations were standardized to express the variety of morphologic changes encountered. Because the field of endeavor was developing rapidly, the nomenclature system was designed in such a way that additional changes could be incorporated as more information became available. The original system has undergone repeated reviews and refinements. The latest review in 1995 has produced a new manual that is extremely clear and usable. A simple review of the system is given here, but it is strongly recommended that for complicated cases the original ISCN 1995 (17) be used.

Use of Symbols and Abbreviations

The normal description of the male and female karyotype was defined as 46,XY and 46,XX, with the modal number given first, followed by the sex chromosomes. Plus and minus signs were to be used to show addition or subtraction of whole chromosomes if placed before the chromosome number and addition or subtraction of parts of chromosomes if placed after the chromosome number. The letters p and q were adopted to designate the short and long arms of any chromosome (p for petit, and q because it follows p). Two forms of karyotype were suggested: the short form listed modal number, sex chromosomes, and any abnormal chromosomes, including break points of translocations; the long form included a sequential description, from one end to the other, of any abnormal chromosomes. Initially, the system was quite cumbersome; in describing abnormalities, missing chromosomes were to be listed with a minus sign before any derivative chromosomes were entered with a plus sign. It is now rec-

ommended that the missing normal chromosomes are not listed in descriptions of translocations where the exchange of abnormal for normal chromosomes is obvious. As an example, the original form for writing the karyotype of a phenotypically normal female with a Robertsonian translocation between chromosomes 13 and 14 was 45,XX,-13,-14,+t(13;14), but it is easier to understand as 45,XX,t(13;14). Some other examples of the simple form of karyotype description, the short system, are 45,X for Turner's syndrome; 46,XY,del(5) for cri-du-chat syndrome; and 47,XY,+21 for Down syndrome.

The presence of more than one cell line in a sample (mosaicism) is indicated by listing both cell lines divided by a slash, such as 45,X/46,XY. The karyotypes are listed with the major cell line first, and the number of cells counted is given in square brackets immediately following the karyotype: 45,X[33]/46XY[17].

Symbols may be used in any combination considered useful. For abnormal rearrangements, the symbol should be placed ahead of the chromosome involved, with parentheses to indicate the extent of the symbol's jurisdiction. For example, 46,XY,r(22) is the correct combination for a male with ring 22, 46,X,i(Xq) for a female with isochromosome Xq (long arm of X). The symbols mat and pat, indicating the origin of a particular derivative chromosome should be given after its description: 45,XX,t(13;14)mat. It is recommended that the symbol mar for marker only be used if the origin of the marker is unknown. The description der is preferred if the original centromere is known.

Nomenclature for Banded Chromosomes

The development of banding meant that every chromosome could be identified individually. The various bands on each chromosome could be described using a numeric system that divided each chromosome into sequential regions. Every band belongs to a specific region, and there are no interbands.

The schematic diagram developed at the Paris conference (1971) is based on fluorescent and nonfluorescent banded chromosomes and shows the major identifying bands of each chromosome (see Fig. 3). Bands are numbered consecutively from the centromere to the terminal ends, upward on the short arm and downward on the long arm. On the diagram, the main regions, which are identified by major, or landmark, bands, are assigned numbers, and the areas within the region are given a second number (Fig. 21). The numbers are written beside the midpoint of the band to which they apply, but the number applies to the whole band. At the region division, the indicated band is band 1 of the distal region. This is not a decimal system, and not all integers are used: band q13 can be adjacent to band q21 if region q1 has only three bands. Provision has been made in the nomenclature system for high-resolution banding; subdivision of main bands into

This chromosome has one landmark band in the q arm, dividing it into two regions.

Bands are numbered outward from the centromere. A two-digit code defines region number and band number.

This numbering system is used when specifying breakpoints; if a break is between two bands, the higher number band, further from the centromere, is listed.

FIG. 21. Regions and bands—numbering system.

subbands is accomplished by the addition of a period and another descriptive number. The period is not a decimal point, and again, not all integers are used: subband p11.2 can be adjacent to subband p12 (Fig. 22).

In summary, to designate a particular band, three items are required: the chromosome number, the arm symbol, and the region number and band number as a two-digit code. These four items are listed in this order, without intervening punctuation. (If the description includes a subband, a period is included before the subband.) Sometimes it is necessary to specify the banding method used. Codes to describe the different methods are given in Table 3. If the codes are used, they are added to the end of the karyotype description.

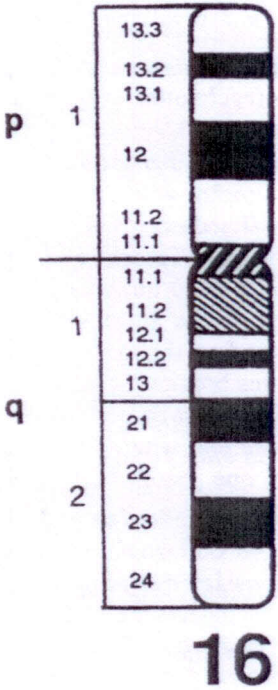
Karyotype Description of Structural Abnormalities

The short system for describing chromosomes is very useful but does not always give sufficient information. When it is necessary to include the detailed composition of any derivative chromosomes formed as a result of translocations and to indicate the breakpoints where translocation segments are joined to each other, the detailed system is used, with descriptions of segments of chromosomes added to the karyotype after the modal number and sex chromosome constitution. The descrip-

tion begins at the terminal end of the short arm, pter, if it is present, and works down through the centromere to the terminal of the long arm, qter, listing any break points as they occur. The centromere need be mentioned only if it is involved in any rearrangement. As an example, consider the pericentric inversion of chromosome 9: in the short system, this is 46,XX,inv(9)(p13q21). In the detailed system it becomes 46,XX,inv(9)(pter→p13::q21→p13::q21→qter). With a terminal deletion of the short arm, as in cri du chat, the detailed description of the deleted chromosome starts at the end of the long arm: 46,XY,del(5)(p15) becomes 46,XY,del(5)(qter→p15:). A single colon is used to indicate a break with no identifiable material distal to it. With broken chromosomes, the ends

TABLE 3. Codes to describe banding techniques

The first letter denotes the type of banding.	
The second letter indicates the general technique.	
The third letter gives the type of stain.	
Examples:	
CBG	C bands by barium hydroxide using Giemsa
GAG	G bands by acetic acid using Giemsa
GTG	G bands by trypsin using Giemsa
GTW	G bands by trypsin using Wright's
QFH	Q bands by fluorescence using Hoechst 33258
QFQ	Q bands by fluorescence using quinacrine
RBG	R bands by BrdU using Giemsa
RFA	R bands by fluorescence using acridine orange
RHG	R bands by heating using Giemsa



This chromosome is divided into regions defined by landmarks and into bands like the chromosome in Figure 21. In addition, sub-bands are indicated by numbers following the decimal point: For example, 16q12.2 indicates chromosome 16, long arm, region 1, band 2, sub-band 2.

FIG. 22. Regions, bands, and subbands—numbering system.

are always capped with a terminal section, but this break may be too small to identify separately.

Specification of Breakpoints

All major bands on chromosomes have identifying numbers. Breaks generally seem to occur at band interfaces. A breakpoint between two bands is given the higher band number (i.e., the number of the band further from the centromere). For example, a break on chromosome 3, in the short arm at the interface between bands 21 and 22, is written as 3p22. To specify the interface absolutely, two zeros may be added, indicating that no part of band 22 is thought to be missing: 3p2200. This method is actually rarely used because one cannot be absolutely certain where the interface is or whether the break is right at the interface.

For breaks within a major band, the most commonly used system is based on the system for specifying high-resolution banding and uses the subdivisions after a period to name a location in greater detail. Band 3p22 is subdivided into three parts—3p22.1, 3p22.2, and 3p22.3. As before, breakpoints are indicated by the larger numbered adjacent band; a break between 3p22.2 and 3p22.3 would be written as 3p22.3.

In the examples that follow, the short system is given first, followed by the detailed system. Usually in a published work, the detailed form is given at the beginning, but the short system is used thereafter, for brevity's sake. There are two kinds of rearrangements: *intrachromosomal*, involving only one chromosome, and *interchromosomal*, involving more than one chromosome.

Intrachromosomal Rearrangements

Deletion, Interstitial

The descriptions of this are 46,XY,del(15)(q12q14) and 46,XY,del(15)(pter→q12::q14→qter). Note that there is no punctuation within the breakpoint parentheses of the short system because both breakpoints are found on the same chromosome. The double colon indicates breakage and reunion of the chromosome missing all or part of q12, all of q13, and possibly part of q14. Note that when a very small deletion is contained within one band, both breakpoints may be the same: (q33q33)

Deletion, Terminal

The short system is 46,XY,del(7)(q32); the detailed description is 46,XY,del(7)(pter→q32:). The cell has a chromosome 7 with the end of the long arm missing from q32 to the q terminal.

Duplication, Direct or Inverted

In the short system, the section that is duplicated is mentioned. In the detailed system, the duplication is described in the banding pattern of the derived chromosome. A direct duplication is inserted with the same orientation to the centromere as the original segment: dup(4)(q31q34) or dup(4)(pter→q34::q31→qter).

In an inverted duplication, the duplicated segment is inserted in the direction opposite to the original one: dup(4)(q34q31) or dup(4)(pter→q34::q34→q31::q34→qter).

It is not necessary to add the symbols *dir* or *inv* because the order of the breakpoints clearly shows the inserted direction of the duplication.

Duplication, with Rearrangement

A rather more complex duplication has been reported for chromosome 8, which also includes a small deletion. The duplicated segment is the distal long arm segment, but it is attached to the short arm, very close to the end. This is not considered an inversion because the segment is still correctly oriented to the centromere. The deletion of the end of the short arm is not mentioned because it is obvious. In the short system, the arrangement is not clearly specified, and the detailed system is needed to understand the composition of the derived chromosome: *rea* dup(8)(q23qter) or *rea* dup(8)(qter→p12::q23→qter).

Fragile Site

A fragile site that has been demonstrated on a chromosome is indicated with the abbreviation, *fra*, and the location. The most well-known fragile site is the site on the long arm of chromosome X, *fra*(X)(q27.3), but other sites are relatively common, e.g., 46,XY,*fra*(16)(q13).

Inversion

Inversions can result from the rejoining of a chromosome after breakage in two places if the internal section is inverted and rejoined upside down (i.e., reversed in relation to the centromere). A paracentric inversion is found within one arm of the chromosome. An example of a paracentric inversion is *inv*(4)(q22q32) in the short system and *inv*(4)(pter→q22::q32→q22::q32→qter) in the detailed system.

A pericentric inversion includes the centromere in the inverted portion and has one break in each arm of the chromosome. The most common pericentric inversion involves chromosome 9, but inversions have also been reported for many other chromosomes. The following are examples of the short and detailed descriptions: *inv*(9)(p13q21) and *inv*(9)(pter→p13::q21→p13::q21→qter).

Isochromosome

An isochromosome is both a duplication and a deletion. The rearrangement involves whole arms of the chromosome, with breakage and reunion within the centromeric region. The band designation for the centromere is p10 or q10. The arm that is present is duplicated, and the other arm is deleted. Isochromosomes have been identified as variations of abnormal sex chromosome constitution and also for some autosomes, particularly chromosome 17, in tumor development. The description *i*(Xq) is equivalent to X(qter→q10::q10→qter). To indicate that in forming an isochromosome both centromeres are included the breakpoints are given as the bands beyond the centromere: *idic*(21q)(p11) or *idic*(21)(qter→p11::p11→qter). Note that the missing sections are not listed.

Ring Chromosome

A ring involves two breaks, usually very close to the terminal ends of the chromosome. The sticky broken ends rejoin in a ring, with loss of the terminal fragments. This is written as *r*(5)(p15p35) or *r*(5)(p15→q35). Note that a colon is not included in this description.

Interchromosomal Rearrangements

Nomenclature becomes a little more complex when two or more chromosomes are involved in a structural rearrangement. If a sex chromosome is involved, it is listed first; otherwise, autosomes are listed in numerical order, and breakpoints are listed in corresponding order. The short system gives the chromosomes involved and the breakpoints for each chromosome. The semicolon is used to separate chromosomes and breakpoints from different chromosomes within each set of parentheses. The detailed system gives the chromosomes involved as in the short form, but it describes the structure of each derived chromosome and separates each derived chromosome with a semicolon. For each derived chromosome, the identity of the chromosome is consistent with the included centromere. For breaks in the long arm, the description can still begin with pter if it is present. For translocations in the short arm, the description must start with qter of the derived chromosome.

Insertion from One Chromosome to Another

In an insertion, a nonterminal section of one chromosome is located between two normally contiguous bands of another chromosome. As with duplicated insertions within one chromosome, these may be either direct or inverted. It is necessary to remember the original orientation of the inverted segment: if the proximal end of the break retains this orientation in the derivative chromo-

some, even if it is moved from one arm to the other, then it is a direct insertion. In some cases the insertion segment may not be defined clearly enough by the banding pattern to determine if it is a direct or an inverted insertion. The recipient chromosome is always written first. (Ideally, the entire karyotype should be written on one line, but the description is frequently so long that it must be split, and this split needs to be located carefully.) An example of a direct insertion is 46,XX,ins(6;10)(q22;q22q24). The segment of 10q, from q22 to q24, is inserted into chromosome 6 at band q22. This gives rise to two derivative chromosomes that are described in the detailed system:

46,XX,ins(6;10)(6pter→6q22::10q22→10q24::6q22→6qter; 10pter→10q22::10q24→qter). An example of an inverted insertion is 46,XY,ins(7;4)(q32;p14p13) or 46,XY,ins(7;4)(7pter→7q32::4p14→4p13::7q32→7qter; 4pter→4p14::4p13→4qter). Note that it is not necessary to state *dir* or *inv*, because the direction of the insertion is given by the order of the breakpoints.

Translocations with Breaks at the Centromere

Derivative chromosomes are the chromosomes that result from a rearrangement of some kind. Usually in a derivative chromosome, the source of the centromere is obvious, but there can be times when it is unclear. Exchanges of whole arms between metacentric or submetacentric chromosomes usually have only one centromere whose source is difficult to determine. For clarity, it is necessary to state which arms are associated with each other. Examples are 46,XY,t(18;21)(18p21p;18q21q) and

46,XY,t(18;21)(18pter→p10::p10→21pter; 18qter→cen→21qter). A slight change is also required in the short system to refer to these derivative chromosomes, which are given as *der*(18p21p) and *der*(18q21q). If the source of the centromere can be determined by staining for polymorphism using C banding or fluorescent techniques, this information can be included in the detailed description: 46,XY,t(18;21)(18pter→18q10::21p10→21pter; 18qter→18q10::21p10→21qter).

Reciprocal Translocation Between Autosomes

In reciprocal translocations between autosomes, in the first set of parentheses, the relevant chromosomes are listed in numeric order and are separated by a semicolon. In the short system the second parentheses have the location of the breakpoints in each chromosome, separated by a semicolon: 46,XX,t(3;5)(q25;q32). The first breakpoint applies to the first chromosome, the second breakpoint to the second chromosome. In the detailed system, the derivative chromosomes are described in numeric order and separated by a semicolon: 46,XX,t(3;5)(3pter→3q25::5q32→5qter; 5pter→5q32::3q25→3qter).

There is no difference if the translocation involves a break in the short arm, except that in the detailed system, the description will start with the terminal of the long arm, qter: 46,XY,t(3;5)(p22;p14) and 46,XY,t(3;5)(3qter→3p22::5p14→5pter;5qter→5p14::3p22→3pter).

Sometimes the breaks are not both in the same arm. The chromosomes should be kept in numeric order, the description of the lowest numbered derivative chromosome should be started first, and the description should begin at the terminal still associated with the original centromere: 46,XX,t(3;5)(p22;q32) or 46,XX,t(3;5)(3qter→3p22::5q32→5qter;5pter→5q32::3p22→3pter).

Nonreciprocal Translocation

It is possible for the broken pieces to reform to make a dicentric chromosome: dic(3qter→3p22::5q32→5pter). However, the other pieces would have no centromere, and although they could join up, they would not be able to attach to the spindle at mitosis and would become lost. This example is therefore not a reciprocal translocation, which has to produce two chromosomes, each with a viable centromere.

Translocation Between a Sex Chromosome and Any Autosome

Such translocation are more likely to involve the X chromosome than the Y. After the modal number, there is only one normal sex chromosome (which may be an X or a Y); the derivative sex chromosome is written next, followed by the derivative autosome. All the other rules apply: 46,X,t(X;2)(q27;p13) and 46,X,t(X;2)(Xpter→Xq27::2p13→2pter;2qter→2p13::Xq27→Xqter)

Robertsonian Translocation

This is a special term that applies to a chromosome formed from the long arms of two acrocentric chromosomes. One or two centromeres may be involved, and this may be determined by fluorescence or C banding. If only one centromere is present, its origin may be impossible to determine and it may be a fusion of parts of both centromeres. Usually the small, satellited short arms become lost. The breakpoint is given as the centromere band for each chromosome, q10. Because the abnormal chromosome is made from two normal ones, the modal number must be reduced by one if the total genetic material is to be normal. The descriptions are 45,XY,t(13;14)(q10;q10) and 45,XY,t(13;14)(13qter→13q10::14q10→14qter). If the centromere is known to be from the chromosome 13 the break points can be written as the bands on either side of the centromere: 45,XX,t(13;21)(p11;q11) and 45,XX,t(13;21)(13qter→13p11::21q11→21qter). Finally, if the

derivative chromosome is dicentric, it can be described as any other dicentric translocation with breakpoints beside the centromere: 45,XX,dic(13;14)(p11;p11) and 45,XX,dic(13;14)(13qter→13p11::14p11→14qter).

It is possible for an abnormality to result from inheritance of a Robertsonian chromosome. Cases of trisomy 13 and trisomy 21 have both been reported with these rearrangements. When an inherited Robertsonian chromosome is associated with an otherwise normal karyotype, one of the normal chromosomes involved in the trisomy is considered to be extra. The chromosomes are written in numeric order, so that for trisomy 13, the description is 46,XY,+13,t(13;14) and for trisomy 21, the description is 46,XX,t(14;21),+21.

Sometimes the Robertsonian translocations are equivalent to isochromosomes being formed from two homologous chromosomes; this has been reported for both chromosomes 13 and 21. When this occurs there is no way to avoid a genetic overdose in offspring. Trisomy 21 with one of these chromosomes is written 46,XY,t(21;21)+21.

Unbalanced Translocation

An unbalanced translocation occurs when only one of a pair of derived chromosomes is inherited. Because the terminal ends of many of the chromosomes have a very similar banding pattern, it can be difficult to determine the source of a small segment of a chromosome. An unbalanced translocation of unknown etiology can be described only as extra material on one chromosome: 46,XX,add(12)(q22). If the translocation is *de novo* and both parents have normal karyotypes, no further identification is possible unless one uses molecular techniques with gene probes.

An unbalanced translocation is frequently the result of inheritance of an unbalanced haploid set from a parent with a balanced translocation. The karyotype can be described in detail, including the composition of the derived chromosome, by referring to the parental karyotype: 46,XY,der(16) t(3;16)(p13;q22)mat. All the chromosomes are normal in this male child except for one chromosome 16, which has a segment of chromosome 3 attached to the long arm. The derived chromosome is der(16)(16pter→16q22::3p13→3pter)mat in the detailed system, and the child is functionally trisomic for the section of chromosome 3.

When the rearrangement is formed from whole arms of the relevant chromosomes with breakpoints at the centromere, it is necessary to clarify which arms are being paired to form the derived chromosomes. Examples are 46,XY,der(18q21q),t(18p21p;18q21q) and 46,XY,der(18),t(18;21)(18qter→18q10::21q10→21qter) for a child who is effectively trisomic for chromosome 21 and monosomic for 18p.

Complex Translocations Involving More Than Two Chromosomes

Multiple breaks and rejoinings during embryogenesis can lead to the development of translocations that are extremely difficult to describe. Each has to be reviewed individually by following the basic rules for simpler translocations and checking frequently with ISCN 1995 (17).

SUMMARY: WRITING A KARYOTYPE DESCRIPTION

Work from left to right and leave no spaces, but separate each item with a comma unless otherwise noted. (By the time everything is described, the karyotype may stretch to several lines; line breaks have to be placed so that the sense of the karyotype can be understood.)

1. Write the number of chromosomes in the cell: e.g., 48.
2. Write all normal sex chromosomes—first the Xs, then the Ys: e.g., 48,XY.
3. Note abnormal sex chromosomes, in this case a dicentric isochromosome of Xq. Precede them with descriptive abbreviation, enclose in parentheses, and follow with breakpoints in a second set of parentheses. The breakpoints must be on the p side of the centromere so that the centromere is included in the isochromosome: e.g., 48,XY,idic(X)(p10),
4. Write the abnormal autosomes or those involved in gain or loss (after a plus or minus symbol), in ascending numeric order, separating the chromosomes with commas. Write the symbol for rearrangement and the chromosomes involved in rearrangement in the first set of parentheses. List them with the X chromosome first, then autosomes in ascending numeric order; separate them with commas. If it is an unbalanced rearrangement, identify the derived chromosome by its centromere: 48,XY,idic(X)(p10),t(5;14),+18,der(20)
5. The breakpoints are added, directly after the chromosomes in a second set of parentheses, with no comma between the two sets. In the short system, the second set of parentheses will contain a list of breakpoints for each chromosome involved in the rearrangement, in the same order as the chromosomes in the first set of parentheses. Remember to indicate which arm is involved as well as the numeric band location. Separate breakpoints with semicolons, e.g., 48,XY, idic(X)(p10),t(5;14)(p11;q22),+18,der(20)t(3;20)(q21;q22.3).
6. In the detailed system, the second set of parentheses should contain the band composition for each derived chromosome, in the same order as given in the first set of parentheses. Start with the p arm if possible, and end at the other terminal. There are three derived chromosomes in this example: 48,XY,

idic(X)(p10),t(5;14)(5pter→5p11::14q22→14qter; 14pter→14q22::5p11→5qter),-18,der(20)(20pter→20q22.3::3q21→3qter).

7. When appropriate, add the origin of abnormal material—mat for maternal, pat for paternal, *de novo* if not seen in either parent: 48,XY,idic(X)(p10),t(5;14)(p11;q22)*de novo*,+18, der(20)t(3;20)(q21;q22.3)mat.
8. If pertinent, add the code for the banding technique used. The finished description is then: 48,XY, idic(X)(p10),t(5;14)(p11;q22)*de novo*,+18,der(20)t(3;20)(q21;q22.3)matGTG.

The verbal description for this fictitious karyotype is a cell with 48 chromosomes, including a normal X and Y, a dicentric isochromosome for Xq, a reciprocal translocation between 5 and 14 not apparent in the parental karyotypes, trisomy 18, and a derived chromosome 20 from a maternal reciprocal translocation of 3 and 20. The trypsin banding was stained with Giemsa stain. Note that without being mentioned, it is clear that there are a single normal 5, 14, and 20, and three 18s. The missing normal chromosomes are replaced by the derivatives.

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